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THE EPIDEMIOLOGY OF WINTER OUTBREAKS OF PARASITIC GASTRITIS IN SHEEP, WITH SPECIAL REFERENCE TO OUTBREAKS WHICH OCCURRED DURING THE WINTER OF 1933-34

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Distribution of the Disease.

DURING the months of January, February, March and April of 1934 a severe epidemic* of parasitic gastritis occurred in the British Isles, principally affecting lambs of the previous year, but in some instances also causing severe losses among ewes and yearling cattle. Within a space of four weeks at the beginning of the epidemic, reports of the unusual prevalence of the disease were received from Lincolnshire, Leicestershire, Buckinghamshire, Kent, Essex, Somersetshire, Herefordshire, Radnorshire, Montgomeryshire, Shropshire and from Northern Ireland. In some districts a considerable proportion of the farmers had sustained heavy losses from the disease, and although Scotland and the North of England appear to have escaped, the total economic loss to the whole country must have been very large.

Extent of the Losses Sustained.

It is not an easy matter to obtain reliable figures on the losses sustained from outbreaks of disease, and particularly from diseases caused by parasitic worms, these being of such frequent occurrence as almost to be accepted by farmers as part of the normal course of events.

* " Epidemic " in the text of this paper refers to the widespread appearance of disease in many different localities ; the word " outbreak " being used to denote the development of the disease in a flock of sheep.

An effort has, however, been made to ascertain the extent of the loss sustained by sheep farmers on the Romney Marsh, where the system of farming appears to lend itself to that purpose. The great majority of the lambs bred in that district are sent away from the Marsh early in September for winter grazing in surrounding parts, and are brought back again early in April; most of the outbreaks occurred while the lambs were away from the Marsh and it therefore appeared to be possible to obtain fairly accurate figures on the number of lambs which died during the winter months. With this aim in view a letter was addressed to the farmers, explaining the desirability of obtaining the information and asking the following four questions:—

(1) How many lambs were sent away by you in September for winter grazing?
(2) How many of these lambs returned in April?
(3) What do you estimate the loss from deaths in £ s. d.?
(4) What do you estimate the loss from poor condition in lambs which have returned, in £ s. d.?

The letter was addressed to 62 farmers on the Romney Marsh and 43 replies were received. The information thus obtained showed that these 43 farmers had sent away 33,373 lambs for winter grazing of which 5,131 died, representing a loss of over 15 per cent. The estimated value of the lambs which died was £7,036 and the estimated loss from the poor condition of many of the diseased sheep which returned was £5,650, making a total of £12,686. During a normal season these farmers allow for an average loss of 5 per cent., from accidents, sporadic disease and also, no doubt, from a certain amount of parasitic gastritis. If, in this instance, a similar allowance is made it is found that these 43 farmers lost £10,341, or an average of £240 each, over and above the usual winter's loss.

There can be no doubt that this extra loss was due to parasitic gastritis. Symptoms and history were typical in every instance where enquiries were made, and at four outbreaks which were personally investigated, *post-mortem* examinations being carried out, a definite diagnosis was arrived at by actual counts of the parasitic worms, which in every instance proved to be over 20,000 worms per sheep.

The figures which have just been given only cover the loss in the lambs sent away for winter grazing by 43 farmers in a very restricted area; and, although the Romney Marsh was one of the most badly affected parts during this particular epidemic, many other parts of the country suffered just as severely and the total loss must have been very great indeed.

The Association between Winter Outbreaks and Prolonged Drought.

Similar epidemics, though rarely as severe as this one, have

previously occurred in the late winter, following a very dry season. The *Monthly Agricultural Reports* of the Board of Agriculture and Fisheries for 1912 show that considerable losses were sustained from " scour " in sheep in various parts during February, March and April of that year, and the *Monthly Weather Reports of the Meteorological Office* show that there had been an unusual drought in many parts of the country during the previous summer and autumn.

The causes underlying the epidemic recorded by M'Fadyean (1897) which occurred in the Eastern Counties in 1895 and 1896 appear to have been somewhat different, as I learn, through the medium of a private communication, that although the outbreak of 1895 was preceded by a rather dry summer and autumn that of 1896 followed a period of heavy rainfall. These epidemics occurred at a much earlier season than the one under discussion in the present paper; of the 1895 epidemic the author writes: " The disease began towards the latter part of September, and broke out almost simultaneously at places widely remote. It continued to carry off large numbers of lambs for several weeks, and declined about the end of October." The 1896 epidemic appears to have occurred at about the same period but mention is made of certain outbreaks occurring in the early part of 1897 also. Although in these latter instances ewes were affected, and carried large numbers of *Trichostrongylus extenuatus* (*Strongylus gracilis*), *Ostertagia circumcincta* (*Strongylus cervicornis*) was principally concerned in the main part of the epidemic.

As clearly shown by the *Monthly Weather Reports of the Meteorological Office* and as generally experienced by everyone, the latter part of 1933 was exceptionally dry and warm; pastures all over the country became very bare, and there can be little doubt that the unusually long period of dry weather was in some way concerned with the widespread occurrence of the disease. An account is here given of enquiries which have been made in an endeavour to ascertain the nature of the factors which operate in prolonged period of drought.

The Nature of the Investigation.

The investigation has been conducted along the lines of an epidemiological enquiry, as discussed in a previous paper (Taylor, 1934b), the histories of individual outbreaks of the disease in various parts of the country having been studied and efforts made to explain the course of events in each instance by information which has been obtained from laboratory experiments.

The epidemiological line of investigation is one which up to the present time appears to have been neglected in the study of parasitic gastritis. The parasites which cause this disease are invariably present in flocks of sheep, and as the *environment* is all important in determining the extent of the multiplication or reduction of their numbers in the susceptible host it is clear that

its influence ought to be the subject of careful inquiry. A considerable amount of work has been done on the influence of external factors upon the development of the infective larvæ of the worms, but that, purely biological, line of enquiry gives little indication of the relative importance of the external factors in the development of parasitic gastritis in a flock of sheep, and from the point of view of preventive medicine, it is most desirable to obtain this information. It is felt that a careful study of the historical epidemiology of the disease as it naturally occurs under farming conditions, in conjunction with the knowledge obtained from laboratory investigations, will throw some light on the development of the disease, and indicate ways in which it may be prevented.

The Nature of the Outbreaks.

The lambs born in 1933 suffered most severely, but in many districts losses were also frequent among the ewes, and several outbreaks were observed among young cattle.

Although the culmination of many of the outbreaks occurred on arable land, the enquiry indicates that the development of the disease has essentially been associated with permanent pasture. The most severe outbreak seen was one occurring in 200 lambs which had been put out to 30 weeks' winter grazing on August 24th and were confined to three grass fields where the herbage was very dry, and later became very short. Up to the end of September the lambs thrived well, but in October they fell away in condition, diarrhœa set in, and in early November they began to die. Up to January 1st 117 had died and when the farm was visited at the end of March, 173 had died, and most of the remaining 27 were in a dying condition. The following worms were collected at a *post-mortem* examination carried out at that date on one of the survivors. From the stomach 30,840 *Trichostrongylus extenuatus,* 10,280 *Ostertagia circumcincta* and 10,280 larval forms; and from the intestine 3,960 *Trichostrongylus vitrinus,* 1,980 *Strongyloides papillosus* and 3,960 larval forms.

This outbreak was the most severe as regards the proportionate loss but it is typical of the rest in the chronic course of its development, this having been one of the most noticeable features of the whole epidemic. Deaths in this outbreak were spread over some five months; but the chronic course of the disease is seen even more clearly in the data presented in the following table, showing the course of the disease to be very different from the usual type of outbreak, seen at the end of the summer. The farmer concerned in this instance kept careful records for the agricultural economics officer and reliance can be placed upon the dates on which the deaths actually occurred.

TABLE I.

Showing the long period over which deaths occurred in a typical outbreak of the disease as it appeared in the period December, 1933 to April, 1934.

Date.	*No. Died.*	*Date.*	*No Died.*
2nd Nov.	1	1st Feb.	3
11th „	1	4th „	1
14th „	1	10th „	2
16th „	1	12th „	2
20th „	1	13th „	1
21st „	2	18th „	2
25th „	2	19th „	2
26th „	1	21st „	2
27th „	1	22nd „	2
1st Dec.	1	23rd „	1
5th „	1	24th „	1
11th „	1	26th „	2
12th „	1	28th „	2
5th Jan.	1	2nd Mar.	3
13th „	2	7th „	1
21st „	1	19th „	1

Total deaths = 47, spread over a period of 4½ months.

The Factors Connected with the Permanent Pasture, of Importance in Producing the Disease.

These will be considered under four headings :—

(1) *The Weather Conditions.*

In the *Summary for the Year* 1933 published in the *Monthly Weather Report of the Meteorological Office*, Vol. 50, No. 13, appears the following : " The year 1933 was distinguished by an exceptional deficiency of rainfall. Other important features of the weather were the marked excess of sunshine from January to March and June to September, the long spell from March to October with temperatures well above the normal."

Mention has already been made of losses from " scour " in sheep in the early part of 1912, and reports have been received from farmers of an epidemic of parasitic gastritis similar to the one under discussion occurring in the winter 1911-12. Weather reports show a drought in the latter part of 1911 and a great scarcity of grass, similar to the grazing conditions of the latter part of 1933. It, therefore, seems very probable that the dry weather has played a major part in the causation of the disease at those two seasons.

The effect of shortage of rain is very noticeable in this country where the pastures are usually moist and green throughout the whole year, and carry a good growth of grass. In dry years the grass ceases to grow and loses its greenness in elevated places with shallow soil, while the ground becomes dry and hard and exceptional grazing conditions result. According to the published laboratory work on the subject such conditions as these should have an adverse influence upon the development of the larvæ,

dryness being considered the most lethal natural factor, and it would seem probable that where the fæces lie on the surface of bare ground and become dry and hard, only a small proportion of the larvæ which may hatch from the eggs can ever reach the infective stage.

It is possible, however, that there is an accumulation of the eggs of the worms on the ground throughout a period of dry weather, and that a subsequent mass development of infective larvæ takes place when suitably moist conditions recur.

The following observations indicate this possibility.

Experiment on the Fate of the Eggs and Larvæ under Drought Conditions.

A preliminary trial showed that eggs in fæcal pellets dried in the incubator at 37° C. retained their vitality for at least seven weeks, but that larvæ were recovered in smaller numbers, and tended to disappear altogether if the pellets were moistened by immersion in water for a few minutes once or twice during that period. It was then decided to make some observations on fæcal pellets under artificial summer conditions in the laboratory.

Fæcal pellets were collected from a sheep carrying a mixed infection of *H. contortus, O. circumcincta, C. curticei, T. extenuatus* and *T. vitrinus* and passing some 1,800 eggs per gram of fæces. They were placed on filter paper, uncovered, in twelve small petri-dishes, four of which were put into the incubator at 37° C., four into a desiccator, also at 37° C., and four kept at room temperature. Each one of the four petri-dishes of pellets in each group was then exposed to artificial dew for seven-hourly periods, at varying intervals; one petri-dish to seven hours' dew in every 24 hours, one to seven hours' dew in every three days, one every five days and one every seven days.

The dew was formed by standing the petri-dishes along with a bowl of hot water under a large glass dish covered with a wet cloth. To help the condensation, and make the conditions equal for each of the different lots, the petri-dishes to be exposed to dew each day were placed in the cold room at about 40° C. for half an hour beforehand. After the first five weeks of the experiment ten drops of water were also placed on the filter paper on which the pellets stood in the petri-dish on each occasion when they were exposed to dew. The dew which formed could not be described as a heavy one but was frequently sufficient to deposit in minute beads on the sides of the petri-dishes.

The viability of the eggs was tested at weekly intervals by placing one pellet from each group along with a few drops of water in a watch glass, and allowing it to stand for 24 hours at room temperature, at the end of which time the water was examined for first-stage larvæ.

The results of the experiment are shown in the following table :—

TABLE II.

Giving the results of the observations on the viability of infection in dried faecal pellets exposed to dew for seven-hourly periods at various intervals. Desiccation is seen to have killed the eggs in two to three weeks, but they are shown to be capable of withstanding air drying for as long as 16 weeks. Those which received most dew, however, and were not dried too quickly (kept at room temperature and not at 37°C.) lost their vitality much more quickly, presumably because the eggs hatched and the preinfective larvae were unable to withstand the dryness.

Conditions under which the faecal pellets were kept.	*Interval at which faeces were exposed to dew.*	*Days after the commencement of the observations at which the viability of the eggs was tested, and the results obtained.*															
		7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112
Air dried at room temperature	Daily	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
	3 days	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
	5 days	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
	7 days	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
Air dried at 37° C.	Daily	+	+	+	+	+	+	+	—	+	+	+	—	—	—	—	—
	3 days	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+
	5 days	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
	7 days	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Dried in desiccator at 37° C.	Daily	+	+	—	—	—	Discontinued										
	3 days	+	—	—	—	—											
	5 days	+	+	—	—	—											
	7 days	+	—	—	—	—											

In no instance were third-stage larvæ recovered from the pellets and it was evidently impossible for development to proceed under the alternating conditions of moisture and dryness. This suggests that during periods of dry weather, even with frequent dews, there is no development of infective larvæ on a bare pasture; although it seems not unlikely that, where the herbage is long, the fæces would retain their moisture for a much greater period, and it is probable that infective larvæ develop. Such condition would, however, be rare on permanent pasture after a prolonged dry period, as the herbage is then short and the fæces are exposed to rapid drying.

The viability of the eggs in the experimental fæces is seen to have been exhausted first in the fæcal pellets exposed most frequently to dew, and in those kept at room temperature before those kept at 37° C., and it seems probable that the moisture resulting from the dew was sufficient to cause many of the eggs to hatch; its greater effect at room temperature doubtless results from the slower rate of drying after the seven hours' exposure to moisture. The maximum survival period was thus seen to be 16 weeks and this occurred in those eggs kept at 37° C. and exposed to seven hours' dew once every week.

An examination carried out on fæcal pellets taken from a field pen after a period of four weeks' dry weather, and three weeks after all sheep had been taken out of the pen, showed that only eggs were present. (Larvæ could not be recovered from them immediately, but after 24 hours numerous first-stage larvæ were obtained.)

Mönnig (1930) made some very interesting and important observations on the reaction of the pre-infective stages of *Trichostrongylus* spp. to dryness. Development of the eggs and larvæ was allowed to proceed to various stages when the fæces, in which they had been cultured, were quickly dried. They were then tested at intervals for viability, when it was found that by far the most resistant stage is that of the egg containing a fully formed embryo. Eggs which have not reached that stage of development, or first- and second-stage larvæ, are easily destroyed by dryness, and even the infective larvæ are considerably less resistant than the embryonated eggs, some of which were found to remain viable throughout 15 months' air drying in a laboratory. Similar observations carried out on *H. contortus* and *Œsophagostomum columbianum* showed that these worms did not possess the same power of resistance and none of the pre-infective stages were able to recover after having once been dry.

Under winter conditions in South Africa it was found, however, that fæcal pellets exposed in the open either dried before the eggs became embryonated or before the larvæ which had been liberated as a result of the occasional rains or dews had reached the infective stage, and it was concluded that *Trichostrongylus* larvæ can only exceptionally reach the infective stage under winter conditions.

Inferences Drawn from the Experiment.

The resistance of the embryonated eggs of *Trichostrongylus*, as demonstrated by Mönnig, has probably been of great importance in the production of the epidemic under discussion. The observations carried out at this laboratory suggest that frequent dews are not sufficient to lead to the hatching of all of the eggs, some of which may retain their vitality for as long as 16 weeks. Dried fæces collected from the pastures showed that infection was lying dormant there and it is clear that a gradual storage of potential infective material accumulates on the ground throughout a period of weeks when drought occurs; and if, at the end of that time there is a suitably long period of wet, warm weather, perhaps a week or more, a mass development of the infective larvæ may presumably take place. If, on the other hand, a long period of drought is followed by a wet period of only one or two days, after which interval dry and hot weather returns it seems probable that the store of potential infective material is destroyed, as the vulnerable first- and second-stage larvæ will then be exposed to dry conditions, which they are unable to withstand.

(2) *The Shortage of Pasture.*

It has previously been pointed out that a scarcity of herbage on the fields where sheep are grazing has a powerful influence upon the intake of infective larvæ. When grass is plentiful the larvæ tend to be left behind as the majority are situated close to the ground, but when the pastures become very bare the sheep crop more closely and on that account take in more larvæ; but, more important than this, they need to graze for a longer period each day in order to satisfy their food requirements, and so collect the larvæ from a greater area.

Jones (1928) made some interesting observations on the relative length of time spent by sheep in grazing on rough pasture and on temporary lay, and found that whereas on the temporary lay lambs spent 49 per cent. of their time in resting, and on good permanent pasture, 45 per cent., on rough pasture they only spent 31 per cent. in resting. Judging by the extreme shortness of pasture in many places in the latter part of 1933 and early in 1934 it seems probable that the sheep would be required to graze for an even greater proportion of their time than those observed by Jones in order to find enough food; and conditions for a maximum intake of infective larvæ must have prevailed.

This increase in the intake of infective larvæ cannot, in itself, have accounted for the heavy infection, however, as it is well known that healthy adult sheep are difficult to infect with some species of the trichostrongylids which cause parasitic gastritis. Mönnig (1929) found it " quite impossible " to infect good conditioned but otherwise perfectly susceptible sheep with *Trichostrongylus extenuatus*, and in all probability the extreme shortage

of pasture has as powerful an action in lowering the resistance of the host as it has in increasing the intake of infective larvæ; this point, however, will be dealt with more fully in the section on immunity.

(3) *The Species of Parasite Concerned.*

The following particulars are of interest in showing something of the species of parasite that have been principally involved and the numbers in which they have occurred :—

TABLE III.

Showing the numbers of the various species of worms which have been recovered from individual sheep in outbreaks occurring in different counties during the winter 1933-1934.

	Somerset.	*Surrey.*	*Kent.*	*Caernarvon.*	*Mont.*	*Lincs.*	*Leics.*
Trichostrongylus extenuatus ...	46,200	1,320	2,400	32,670	21,840	9,520	58,760
T. vitrinus ...	51,300	6,550	28,800	44,980	4,680	34,370	20,790
T. colubriformis	—	5,240	3,610	—	—	—	7,560
Ostertagia circumcincta	—	120	8,400	11,880	11,760	6,800	—
Nematodirus filicollis ...	—	—	—	—	1,800	7,365	—
Cooperia curticei ...	—	—	—	—	360	2,450	—
Hæmonchus contortus ...	—	200	—	—	—	—	—
Larvæ	—	3,580	4,810	39,070	360	15,790	—

These represent only a small proportion of the number of outbreaks which have been investigated but serve to show the similarity of the helminth infestation occurring in various counties. The parasites principally concerned in the series of epidemics which occurred during the period under consideration are thus seen to belong to the genus *Trichostrongylus*, the species *T. extenuatus* and *T. vitrinus* being particularly involved; in some instances, however, *Ostertagia circumcincta* was also represented in large numbers.

Species of the genus *Trichostrongylus* appear only infrequently to cause disease in Great Britain; although, in small numbers, they are almost invariably associated with other species wherever outbreaks of parasitic gastritis occur.

(4) *The Susceptibility of the Sheep concerned in the Epidemic.*

The relative resistance of adult sheep to parasitic gastritis is well known to all who have to do with the rearing of sheep. Outbreaks are frequently observed where lambs and ewes have been exposed to the same infection throughout the whole life of the lambs, but the ewes remain perfectly healthy, while the lambs die of the disease. Stoll (1929) traced the development of this resistance by following the variations in the number of eggs passed

in the fæces of lambs infected with *Hæmonchus contortus* and grazing on a limited amount of pasture. He was satisfied that the immunity of his lambs had been acquired as a result of the infection, although it is not possible to disregard age immunity in the interpretation of his results.

In 1934 Taylor (1934a) reported a demonstration of this acquired immunity, and interpreted the results which he obtained as indicating that the immunity operates more powerfully on *Hæmonchus contortus* and *Nematodirus filicollis* than on *Ostertagia* spp., *Trichostrongylus* spp., and *Cooperia* spp. His resistant lambs were free from *Hæmonchus* and adult *Nematodirus* (although several of them carried larval *Nematodirus*), but all carried heavy infestations of *Ostertagia, Trichostrongylus* and *Cooperia*. Morgan (1933) reported a similar observation in some experimental goats grazing on limited pasture; *Hæmonchus contortus* larvæ were seen to be fairly numerous in cultures made early in the summer but very few adults were found when the *post-mortem* examinations were held in December. He appears to have been of the opinion that this difference was to be accounted for by biological differences in the early development of the free living larvæ. Mönnig (1929) reported that he was unable to infect healthy but otherwise susceptible sheep with *Trichostrongylus extenuatus* unless they were definitely undernourished, and that the sheep which became infected under those conditions quickly eliminated their parasites when placed on an adequate diet.

Fraser and Robertson (1933) have recently published a paper on the influence of the nutritional state of sheep upon their susceptibility to infestation with *Hæmonchus contortus*, in which they give an account of their own observations on the *H. contortus* infestation of a well fed group of lambs as compared with that of a poorly fed group. The infestations with which they dealt were, however, very light, averaging only 31 parasitic worms per sheep in the well fed group, and 103 per sheep in the poorly fed group, so that the question of disease did not arise, some ten to twenty thousands of worms apparently being necessary to cause any marked disturbance of health.

The previous work on the influence of diet upon infestation with parasitic worms has been mentioned in Fraser and Robertson's paper, and it will not be necessary to repeat it here, but only to describe the experiments which have been carried out at Weybridge to ascertain something of the influence of this factor upon the development of parasitic gastritis in sheep.

Experiment No. 1.

Demonstration of the Influence of Low Diet upon the Process of Infestation with Trichostrongylids.

Six lambs, between seven and eight months' old, which had been confined to a one-third acre plot, where unsuccessful efforts had been made to set up parasitic gastritis with a pure infection of *Ostertagia circumcincta*, were brought

into the farm buildings on October 27th, 1933, and divided into three pairs, one pair being fed on a liberal ration of hay and concentrated food, one pair on hay only, and one pair on straw only. When brought into the farm buildings each lamb carried a very light infestation of some trichostrongylids but what species they were is not known except that the eggs of *Nematodirus* appeared along with the *Ostertagia* and *Hæmonchus* type in some of the fæces. Between the end of October and the beginning of January each lamb received 40 doses of 500 infective larvæ of trichostrongylids of various species, from a culture made from the fæces of a sheep infected with *Hæmonchus contortus*. *Trichostrongylus* spp., *Ostertagia circumcincta* and *Cooperia curticei*. Egg counts were made on the fæces of these lambs at various times during the course of the experiment, and at the end (March 14th) the lambs were killed and an estimation made of the number of worms which they carried. The results obtained are set out in the following tables :—

TABLE IV.

Showing the number of eggs per gram of faeces on various dates throughout the experimental period, in sheep which received the same number of infective larvae but were fed on different diets.

No. of lambs.	*Nature of food.*	*Number of Eggs per gram of Fæces.*								
		Dec. 11th.	*Jan. 5th.*	*Jan. 29th.*	*Feb. 7th.*	*Feb. 14th.*	*Feb. 21st.*	*Feb. 28th.*	*Mar. 7th.*	*Mar. 14th.*
1	Hay and cake	400	100	300	50	250	100	100	100	300
2		900	1,700	2,300	2,300	2,400	1,750	3,100	1,300	800
3	Hay only	2,100	2,900	6,500	4,400	6,750	7,450	4,450	4,400	2,500
4		2,400	3,900	8,000	5,350	5,350	5,100	4,550	5,450	2,100
5	Straw only	2,400	4,100	5,500	5,200	10,000	5,000	6,000	3,650	2,850
6		3,400	4,600	9,000	8,350	10,050	7,500	7,800	—	—

Note.—The special feeding of these lambs and the dosing with infective larvæ began on October 27th.

It is thus seen that the sheep receiving straw only harboured over three-and-a-half times as many worms and passed about five times as many eggs as did those which received a full diet, while those fed on hay only, occupied an intermediate position.

Experiment No. 2.

Demonstration of the Influence of Low Diet upon the Normal Elimination of the Parasites.

A second experiment of a somewhat similar kind shows the effect of an inadequate diet in inhibiting the normal elimination of these parasites from the infected sheep. Six lambs, aged eight to nine months and carrying a more or less heavy mixed infection of trichostrongylids were brought into the buildings from the infected field pens. They were divided as evenly as possible into two groups ; the first group were then fed on a liberal ration of hay and cake, and the second fed on hay only. Counts were made each week of the numbers of eggs per gram of fæces from each lamb, and the weights of the lambs were taken at the same time. The results are set out in Table VI, page 248.

TABLE V.

Showing the numbers of worms recovered at *post-mortem* examination from sheep which had received the same number of infective larvæ but for five months had been fed on different diets.

No of lambs.	*Nature of food of lambs.*	*Hæmonchus contortus.*	*Ostertagia circumcincta.*	*Trichostrongylus extenuatus.*	*Trichostrongylus vitrinus.*	*Trichostrongylus colubriformis.*	*Cooperia curticei.*	*Total Worm count.*
1	Hay and cake	200	100	—	24	—	24	348
2		200	504	196	2,320	464	2,784	6,468
3	Hay only	1,500	400	400	2,844	—	3,634	8,778
4		1,400	928	672	1,768	272	4,760	9,800
5	Straw only	700	754	2,088	3,444	—	4,814	11,800
6		1,100	816	2,584	1,880	752	6,768	13,900

TABLE VI.

Showing the weights of the lambs in lbs. and the numbers of eggs per gram of fæces on successive weeks in two groups of lambs, the first being fed on an adequate diet, and the second an inadequate diet.

	On a full diet of Hay and Cake.						*On an inadequate diet of Hay only.*					
	Lamb No. 710.		*Lamb No. 708.*		*Lamb No. 702.*		*Lamb No. 656.*		*Lamb No. 659.*		*Lamb No. 648.*	
	Wt. in lbs.	*Eggs per gram.*	*Wt. in lbs.*	*Eggs per gram.*	*Wt. in lbs.*	*Eggs per gram.*	*Wt. in lbs.*	*Eggs per gram.*	*Wt. in lbs.*	*Eggs per gram.*	*Wt. in lbs.*	*Eggs per gram.*
Dec. 14	62	1,850	86	100	92	300	61	2,350	88	650	86	700
„ 21	56	3,850	91	250	89	400	59	2,050	88	150	84	1,950
„ 28	55	1,700	90	500	90	150	59	1,150	59	1,050	86	1,650
Jan. 4	55	1,950	90	50	92	500	62	1,250	85	250	78	2,350
„ 11	48	1,300	88	50	89	150	59	1,400	84	600	76	2,400
„ 18	49	2,300	90	100	89	200	56	1,700	86	650	76	1,200
„ 25	48	3,500	88	100	90	150	62	2,950	80	0	71	2,900
Feb. 1	44	2,550	94	200	93	0	59	2,050	74	2,350	74	2,850
„ 8	44	4,350	92	0	93	150	57	2,250	74	1,100	66	2,450
„ 15	45	3,200	98	100	95	600	56	2,800	73	1,600	65	1,500
„ 22	43	3,100	100	100	98	0	58	3,150	78	1,650	66	2,300
Mar. 1	43	3,800	101	50	103	0	58	2,400	74	1,400	66	1,600
„ 8	43	3,100	102	50	107	0	56	1,450	70	2,050	68	1,200
„ 15	42	3,700	105	200	110	50	56	3,200	72	1,000	68	2,100
„ 22	44	1,500	110	50	115	50	54	2,300	70	1,550	69	2,250
„ 29	45	1,450	114	0	120	0	Killed when		74	1,000	72	1,650
Apr. 5	44	1,500	116	50	124	50	in a		72	1,350	62	2,300
„ 12	46	1,550	119	0	129	50	very weak		73	1,500	74	1,400
„ 19	48	1,500	124	0	132	0	state.		73	2,450	72	1,900
„ 26	49	1,300	126	100	124	50			79	1,900	77	1,050
May 3	46	1,400	126	50	124	0			81	2,650	76	1,700
„ 10	50	500	135	50	128	0			79	2,250	76	1,800
„ 17	50	950	137	250	129	50			83	3,300	78	1,950
„ 24	50	500	138	50	127	0			78	4,050	77	3,550
„ 31	54	450	140	100	128	300			82	3,000	78	5,100
June 7	54	600	132	50	121	100			80	3,900	77	6,800
„ 14	—	650	0	0	—	100			—	4,200	—	6,600
„ 21	60	850	142	0	132	0			80	5,650	80	7,400
„ 27	60	600	140	0	128	100			67	5,850	68	11,700
July 4	64	200	143	0	130	50			72	5,400	76	10,650
„ 11	65	550	143	0	128	100			75	5,200	72	6,950
„ 18	68	500	144	0	127	50			72	6,900	76	17,950
„ 25	72	1,000	150	50	130	50			78	9,900	75	15,200
Aug. 1	76	850	148	50	130	50			74	4,200	74	18,100
„ 8	75	1,100	148	0	130	100			72	4,450	72	15,950
„ 15	80	1,600	150	0	134	0			75	4,350	72	15,700
„ 22	81	1,350	150	50	132	50			74	5,500	74	16,250
„ 29	82	500	157	50	130	100			73	5,450	75	25,400

As the number of eggs passed by the lambs which were receiving a ration of hay only actually increased throughout the nine months observational period, it was naturally supposed that they were picking up infection in the indoor pens, although these were thoroughly cleaned out twice every week. A worm-free lamb was, therefore, placed in the pen along with the others on May 28th and a flotation test carried out on the fæces every week ; the results were, however, completely negative up to July 4th, when only one egg was recovered, by flotation, from 4 grams of fæces.

This lamb was then taken out to be used in another experiment and a second worm-free lamb placed in the pen. Up to August 29th, floatation examinations of the fæces of this lamb have given negative results, and it, therefore, seems unlikely that the experimental lambs had been acquiring infection while in the indoor pens, and the increase in the egg output of the worms is probably to be explained by a gradual lowering of the resistance of the lambs which carried them.

Discussion on the Experiments on the Effect of Diet.

These experiments show the marked influence of the nutritional state of the sheep upon the process of infection with these parasitic worms, and upon their persistence in the sheep. In the first experiment the lambs receiving a full ration were found to contain the fewest worms and those receiving straw only to contain the most. Differences due to individual susceptibility, which have been observed in several experiments with these parasitic worms, are in evidence in the first pair, and it is clear that caution must be observed in drawing conclusions from so small a number of experimental animals, but it is strongly suggested that an inadequate diet exerted a marked influence in breaking down the resistance of the sheep to the development of the parasitic worms. In view of the results of the second experiment this interpretation of the first is rendered even more probable. The egg output of the three heavily infected lambs placed on a liberal diet in the second experiment decreased throughout the seven months' observational period to about one-ninth the original number. Apparently they were able to eliminate their parasites quite successfully. In the other three, however, receiving an inadequate diet, the egg output actually increased to about five times its original number, probably as a result of a slow breaking down of the resistance of the lambs and that part of immunity which inhibits egg production; they remained in a chronic condition of extreme debility, gradually worsening throughout the seven months' observational period, and no elimination of their parasitic worms appears to have taken place.

Morgan (1933) noted something similar in a series of observations on the numbers of eggs passed in the fæces of lambs grazing on a limited area of pasture. He found a slight increase to take place throughout the summer and a marked increase in October, coinciding with the deterioration in the quality and quantity of the herbage at that time of the year when all his experimental plots had become rather bare.

Conclusion Drawn from the Experiments on the Effect of Diet.

It may, therefore, be concluded that a deficient diet lowers the resistance of sheep to infection with the worms which cause parasitic gastritis, and interferes to a marked degree with the elimination of an infestation already present.

It is now pertinent to consider the diet of the sheep which suffered from parasitic gastritis in the winter of 1933-34.

Nutrition of the Sheep Concerned in the Epidemic.

Some interesting work has been done by Stapledon, Jones and co-workers at the Welsh Plant Breeding Station on the effect of various pastures upon the growth of the sheep, which may have some bearing upon the development of outbreaks of parasitic gastritis such as the one under discussion. These points are as follows: The blade of the grass is known to be much more nutritious than the stem, and Jones (1928) in the paper which has previously been mentioned points out that the percentage of stem increases with close grazing. He observed, in comparing the results of his trials with the results of similar ones carried out by Stapledon and Jones (1927) that under relatively dry conditions sheep are able to graze much less equivalent of air dried matter from the pasture per day than they do under relatively moist conditions, when the herbage is more succulent the actual figures being 2·6 lbs. and 3·4 lbs. per head per day respectively. Furthermore, it is well known that the nutritive value of the grass decreases very markedly towards the end of the summer. Jones (1928) found the June growth of grass to produce an increment of 5·6 lbs. of mutton per acre per day, the July grass an increment of 4·7 lbs. and the August grass an increment of only 2·1 lbs. of mutton per acre per day. Stapledon *et al* (1927) found that burnt herbage is poor in protein, silica-free ash and mineral constituents, and relatively rich in fibre.

The grazing afforded by the very short, dry herbage of the pastures during the latter part of the summer and right on throughout the winter appears, therefore, to have been of a poor quality, greatly inferior to what is usual, and it seems highly probable that in consequence the sheep were actually under-nourished, and as a result their resistance to parasitic invasion became greatly lowered.

General Discussion.

It is thus shown that during the months December, 1933, to April, 1934, there has been a widespread epidemic of parasitic gastritis in sheep in many parts of Great Britain, excluding Scotland and the North of England. That it has been caused almost entirely by species of *Trichostrongylus* and *Ostertagia* (particularly by *Trichostrongylus extenuatus* and *T. vitrinus*) and that *Hæmonchus contortus* and *Nematodirus filicollis* have played little or no part. Weather conditions prior to and during that period have been exceptionally dry; reports indicate that a similar outbreak occurred following a similarly dry period in 1911, and it appears probable that dry weather is an important epidemiological factor where this particular kind of outbreak is concerned.

Comparisons which have been made by other investigators between various kinds of pasture indicate that dried pasture is less nutritious than moist pasture, that sheep cannot graze so much equivalent of air-dried matter where the herbage is dry as they can where it is more succulent, that the leaf of the grass is much more nutritious than the stem and that the proportion of stem is greater where the grass is short; that where pastures are poor the amount of time required daily for actual grazing is greatly increased—requirements for basic metabolism being thereby also increased—and finally, that towards the end of the summer the nutritive value of the grass becomes greatly depreciated.

It is evident that all these adverse influences came into play to a marked degree from the end of the summer, 1933, and onwards throughout the winter, and it seems reasonable to suppose that many of the sheep were under-nourished.

The experiments here recorded show that the resistance of sheep to a mixed infection of the worms which cause parasitic gastritis is greatly reduced when the sheep are placed on a low diet, and it is probable that this factor played an important part in the development of the epidemic.

The observations of Mönnig show that the eggs of *Trichostrongylus* containing embryos ready to hatch are able to withstand up to 15 months of continuous dryness. Exposure to a brief interval of wet weather during a dry period, however, results in their death, as the eggs then hatch and the pre-infective larvæ which are liberated are unable to withstand the dryness. In the observations recorded in this paper it was found that even where exposed to occasional dews the infection remained alive in the fæces up to 16 weeks, the moisture from the dew being insufficient to lead to the immediate hatching of all the eggs. It is clear, therefore, that there are great potentialities here for the storage of infection throughout a period of drought and a mass liberation of infective larvæ when suitably moist weather conditions recur, and persist throughout a long enough period for their development to take place. This is a factor which, at times, may be of the greatest importance in the production of outbreaks of parasitic gastritis by *Trichostrongylus* spp. and possibly by others. If, however, short moist-weather periods of only two or three days' duration occur during a long period of drought, the store of potential infection is presumably killed as there will not be sufficient time for the first- and second-stage larvæ to develop to the more resistant third stage before the fæces (in which these early stages remain) become dried, and the larvæ die.

The sudden appearance of many outbreaks of this disease within a very short period at the beginning of the epidemic suggests that this storage factor may have played a major part in the production of the disease, although a careful comparison of the records

published in the *Monthly Reports of the Meteorological Office*, with the exact dates of the development of various outbreaks of the disease, has not provided any convincing evidence that that factor has been of first importance in the production of the epidemic which is being discussed.

The other factors probably worked in the following way towards the multiplication of the parasites and the propagation of the disease: As a result of the prolonged drought the grass became very short during the summer months, and so gave rise to conditions suitable for a maximum intake of the infective larvæ of the parasitic worms. The poor nature of the herbage and the expenditure of excessive energy in grazing it resulted in an undernourished condition of the sheep and a consequent breakdown of their immunity. The unusually chronic nature of the disease which developed is probably to be accounted for by the action of the prolonged drought in retarding the development of the infective larvæ, or inhibiting it altogether for several weeks at a time. During this period the infection of the sheep would not increase, or if it did so, would only increase slightly. The deficient diet would, however, have a marked adverse influence upon the elimination of the parasitic worms by the sheep, so that their infestation would not decrease (as it, presumably, would do at ordinary times when the rate of intake of infective larvæ became lowered for any reason), but would remain at a level. If that level was a moderately high one it would be sufficient to lower the condition of the sheep gradually, and produce chronic disease—as it was seen to do in the experiment described in this paper.

The last unusual feature of the epidemic to be mentioned is the scarcity or absence of *Hæmonchus contortus* and *Nematodirus filicollis*, which two species are usually present in large numbers in ordinary outbreaks of the disease in England. M'Fadyean (1897) records that he was able to find *H. contortus* in only eight of the 39 *post-mortem* examinations which he carried out in his investigation of the 1896 epidemic. No mention is, however, made of *N. filicollis*. An explanation of this occurrence is suggested in the results obtained by Taylor (1934a), which point to the greater resistance of adult sheep, or of sheep approaching maturity, to *Hæmonchus* and *Nematodirus* than to *Trichostrongylus*, *Ostertagia* and *Cooperia*.

It cannot be concluded as a result of this investigation that all winter outbreaks of parasitic gastritis originate in the factors which are believed to have operated so powerfully during the winter of 1933-34; the probabilities are that totally different weather conditions sometimes bring about the same result through the agency of different factors. It seems highly probable, however, that drought has been the deciding condition in this instance, and if the deductions are correct, a most important step towards the prevention of this type of outbreak appears to be the provision of

more adequate nutrition for the sheep by liberal feeding with concentrates as soon as there is any threat of a scarcity of pasture. Where rainfall permits, great benefit might also be derived from the application of nitrates to some of the pastures during the later part of the summer to stimulate a better growth of grass at the period at which it is usually scarce.

Summary and Conclusions.

The epidemic of parasitic gastritis which has caused such severe losses during the winter of 1933-34 has principally been associated with heavy infections of species of *Trichostrongylus*, but species of *Ostertagia* have also been numerous in some instances. *Hæmonchus* and *Nematodirus* do not appear to have been involved, rarely being represented at all and then only in small numbers.

Enquiries on the Romney Marsh showed that 43 farmers lost £10,341 during the outbreak.

The progress of the disease has been unusually slow, deaths in many outbreaks being spread over four or five months.

The outbreak was associated with a prolonged period of drought, most unusual for this country.

Observations show that infection remains alive for ten weeks in fæcal pellets air dried at room temperature and occasionally exposed to dew; and at 37° C. the vitality is retained for 16 weeks, the dew presumably not providing enough moisture for the immediate hatching of the eggs, which, in the case of *Trichostrongylus*, as shown by Mönnig (1930), are very resistant when just ready to hatch.

A gradual storage of potential infective material probably takes place on the ground during dry weather, which may or may not terminate in a mass development of infective larvæ, depending upon the advent of a period of damp weather of sufficient duration for the infective larvæ to develop.

An experiment on two sheep fed on hay and cake, two fed on hay only, and two fed on straw only, all receiving the same dose of infective larvæ, showed that more worms develop in sheep fed on a deficient diet than in those receiving a full ration.

An experiment on six infected lambs brought into indoor pens, where three were fed on hay and cake and three fed on hay only, showed that those on the adequate diet eliminated their worms, but that throughout the seven months' observational period no elimination occurred from those lambs fed on hay only.

The work of other investigators has shown that short herbage at the end of the summer is of a very poor quality. After a period of drought it is also very scarce and it is probable that during a dry summer many of the sheep are definitely under-nourished.

It is concluded that the principal factors concerned in the development of such a series of outbreaks of parasitic gastritis as

has taken place during the past winter months are the shortage of pasture, leading to maximum intake of infective larvæ, and the poor nature of the pasture, leading to actual under-nourishment of the sheep. The mass liberation of infective material stored up in the dried fæces has also in all probability played an important part.

It is advised that where a shortage of pasture is threatened the sheep should receive an ample allowance of concentrated food and that use might be made of the application of nitrates to some of the pastures towards the end of the summer, to stimulate the growth of grass of a more nutritious quality.

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PULPY KIDNEY DISEASE (ENTERO-TOXÆMIA) OF SHEEP IN PALESTINE

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The occurrence of deaths in sheep which drew attention to the possible existence of either pulpy kidney disease or lamb dysentery in Palestine was first observed in an outbreak at the Jewish settlements of Tel-Yo'sef and Beit Alfa. These flocks consist of the native fat-tailed type in which an attempt is being made to effect improvement by selection.

Deaths commenced at the end of May this year and continued until June 15th, when the sheep were withdrawn from pasture. During this period out of a total of 805 animals 49 succumbed, the ages being approximately as follows:—

6 months	13 died.
1 to 1½ years...	27 „
Aged	9 „

Material forwarded for laboratory examination for anthrax proved negative, but from a piece of spleen an anærobic organism was recovered which proved to be highly pathogenic to guinea-pigs. A sick sheep had also been requested, but, owing to the rapidity with which death took place following the onset of symptoms, a recently dead animal was brought instead to the laboratory by car, a journey of some five hours. From this carcase contents of portion of the ileum, washed out with four times the volume of saline, were filtered through a Seitz filter. Ten c.c. of the filtrate injected intravenously caused the death of a lamb after two hours.

The small intestine from which the filtrate was obtained showed severe inflammation and it was reported by the local veterinary surgeon that such inflammation was present in some 50 per cent. of the fatal cases.

The sheep were then removed from pasture and the mortality ceased, and so it was not possible at this time to obtain further material. The remainder of the filtrate, however, kept at low temperature proved stable for a period of two to three months.

Control experiments on the intravenous inoculation of a sheep with filtrate of normal intestinal contents of sheep proved negative.

Of the original filtrate 0·5 c.c. inoculated intravenously into a rabbit killed in three-quarters of an hour. As mice were not easily procurable, young white rats were used and on these the following experiment was made to ascertain the m.l.d.

75 grammes rat	0·2	c.c.	intravenously	died in 4 hours.	
55 „ „	0·05	c.c.	„	„ 26	„
65 „ „	0·02	c.c.	„	lived.	

Cultures of *B. welchii* (Types A, B, C, and D) and also B and D antitoxin were obtained through the kindness of the Director of the Institute of Animal Pathology, Cambridge. Neutralisation experiments on the filtrate were then carried out as follows :—

90 grammes rat	0·3 c.c.	toxin	+	0·3 c.c.	serum	B —	Lived.
90 „ „	0·3 c.c.	„	+	0·3 c.c.	„	B —	„
80 „ „	0·3 c.c.	„	+	0·3 c.c.	„	D —	„
90 „ „	0·3 c.c.	„	+	0·3 c.c.	„	D —	„
75 „ „	0·2 c.c.	„	(control)—Died 4 hours.				

As, according to Wilsdon,[1] antitoxin D does not neutralise so many m.l.ds. of toxin B, diagnosis of the death of the sheep as due to *B. welchii*, type D, was considered to be sufficiently established.

The filtrate was shown to be thermolabile in the following experiment. Filtrate heated at 60° C. for 15 minutes :—

Rat 80 grammes	0·66c.c.	intravenously	—	Lived.
Rat 45 „	0·5 c.c.	„	—	„
Rabbit	0·5 c.c.	„	—	„
Control rat	0·2 c.c.	„	—	Died $\frac{3}{4}$ hour.
Control rabbit	0·5 c.c.	„	—	„ 4 hours.

A further outbreak was now reported, in July, from the settlement of Mishmar Haemek, situated in the same valley some 20 miles distant. A portion of intestine was received and filtrate from the contents was similarly shown to be neutralised by antitoxin D while 1 c.c. killed a control rabbit. Fresh cultures were also obtained from this intestine.

By this time antitoxin had been prepared from *B. welchii* type A (Cambridge) and preliminary experiments showed that it also neutralised the filtrate when used in equal quantities (0·3 c.c. of each) but not when half the quantity of antitoxin was used. Wilsdon[1] found, however, that 0·2 c.c. of A antitoxin neutralised 2 m.l.d. of D toxin whilst 0·1 c.c. of D serum neutralised 40 m.l.d. of the same toxin. The following experiment was therefore carried out to ascertain the relative antitoxic value against the filtrate of A and D antitoxin.

Rabbit	0·5 c.c. filtrate	— Died $2\frac{1}{2}$ hours.
Rabbit	0·1 c.c. „	— Lived.

Taking 0·25 c.c. filtrate as the probable m.l.d. inoculations were performed as follows :—

Rabbit 8	m.l.d.	+ 0·1	D Cambridge)	antitoxin	—	Lived.
Rabbit 8	„	+ 0·1	D (Laboratory)	„	—	„
Rabbit 8	„	+ 0·1	A	„	—	Died 55 minutes.
Rabbit 16	„	+ 0·1	D (Cambridge)	„	—	Lived.

Culture Experiments.

Microscopical examination of a piece of spleen forwarded from a dead sheep showed large numbers of bacilli morphologically identical with *B. welchii*. 0·3 c.c. of this material was inoculated intramuscularly into guinea-pigs, which died within 15 hours.

The bacillus was obtained in pure culture on isolation from a femur.

Toxin experiments from cultures were next performed, the toxin being obtained both by filtration and also by centrifuging culture and using the supernatant fluid. The following amounts were inoculated intravenously :—

Lamb 5 c.c. — Lived.
Rabbits 0·5 to 3 c.c. — „

This absence or low production of toxicity was at first confusing but it was then observed that the pH. of the medium (Heubler's brain and liver broth) fell in some eight to ten hours from 7·4 to 4·5. The same acidity was also produced in V.F. broth but to a less extent. Fresh tubes each containing 10 c.c. of Heubler's broth were adjusted to pH. 9·0 and inoculated from a stock culture tube. After eight hours the tubes were found to be acid and it was necessary to add 0·3 c.c. of N/1 NaOH to each tube to re-adjust the reaction to pH. 8·4. Some of the tubes were left without readjustment as controls.

On the next day the following inoculations were made :—

Toxin from readjusted tubes.
2 rabbits, 3 c.c. each intravenously — Died in 6 hours.
Toxin from control tubes.
Rabbit, 3 c.c. intravenously — Lived.

The foregoing experiment indicated that the production of acidity by the bacilli interfered with toxin production. In order to maintain the media alkaline, a pipette inserted through the plug was so arranged as to deliver a certain quantity of sterile NaOH into the media in a given time. By means of this device toxin of a much higher potency was produced, 1 c.c. killing a rabbit in six hours and 0·2 c.c. a rat in ten hours. Control tubes without added NaOH required 0·6 c.c. to kill a rat of 55 grammes, whilst 4 c.c. failed to kill a rabbit. Much the same results were obtained on using V.F. broth, except that toxin production was to a slight extent stronger.

The same effect was observed when large quantities, *viz.*, 500 c.c., of media were used, a rabbit inoculated intravenously with 6 c.c. of culture toxin from unadjusted media remaining alive, whilst a rabbit which received 4 c.c. of toxin from adjusted media died in four-and-a-half hours.

The whole experiment was repeated from cultures of *B. welchii* freshly isolated from intestine in the Mishmar Haemek outbreak, and exactly similar results were obtained.

For purposes of comparison a similar culture of *B. œdematiens* was also tested, but although the culture rapidly became acid there was no apparent interference with toxin production, in the single experiment made.

The relation between alkalinity and toxin production suggests that the healthier lambs affected in the field would be more likely

to succumb owing to a tendency in a physiologically healthier organism to correct an unnatural acidity, and this was found to be the case in the present outbreak. It is also stated by Gill[2] that the best lambs are affected in his description of the disease in New Zealand. In a small number of tests the pH. of normal intestinal contents (ileum) of sheep was found to vary from 7·6 to 8·4.

Experiment on the neutralisation of culture toxin from adjusted media resulted as follows :—

Rat 100 grammes 0·5 c.c. toxin + 0·5 c.c. sheep (Laboratory) antitoxin — Lived.

Rat 90 grammes 0·5 c.c. toxin + 0·5 c.c. D (Cambridge) antitoxin — Lived.

Rat 85 grammes 0·3 c.c. toxin (control) — Died 5½ hours.

Culture Reactions.

As cultural reactions at present do not appear to be of great value in diagnosis they were not extensively investigated. In cooked meat medium rapid growth took place with profuse gas production.

Some cultures were also incubated in a McIntosh and Fildes anærobic jar (Wilson's modification), the hydrogen being provided from a cylinder. Incubation was continued for 72 hours with results as follows :—

	Gelatin.	*Inspissated horse serum.*	*Blood agar.*
Beit Alfa strain	Complete liquefaction	Slight liquefaction	Good growth Diffuse hæmolysis
Mishmar strain	„ „	„ „	„ „

From the foregoing experiments it is evident that pulpy kidney disease (entero-toxæmia) of sheep exists in the plain of Esdraelon of Palestine. It is probably common to all sheep in that area at least, including those of the native villages, but had not hitherto been reported there. Mortality appears to occur in spring and early summer. Vaccine and antiserum have been prepared but, as the sheep have now been returned to pasture and there is no resulting mortality, field investigation as to the value of these products will have to be deferred until next year.

The writer wishes to express his thanks to Mr. L. Smerkiss, Chief Laboratory Assistant, for his care and assistance in carrying out the necessary experiments.

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FOOT-AND-MOUTH DISEASE: THE FIXED VIRUS TYPES

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In 1922 Vallée and Carré were able to distinguish two immunological types of foot-and-mouth disease in cattle, now known as "O" and "A."

With the discovery that the immunological difference between these types could readily be demonstrated by cross-infections in guinea-pigs, a good deal of effort was expended on the task of determining to what types the viruses responsible for spontaneous outbreaks of the disease in cattle in various parts of Europe and America should be assigned.

In 1926 Waldmann and Trautwein added a third strain, type "C," to the two already known.

For some years after attention had first been drawn to the suitability of the guinea-pig for this particular work it seems to have been generally accepted that "typing" in guinea-pigs was the only reliable method of determining to what type a bovine strain of virus belonged. With very few exceptions any unusual immunological behaviour in a virus during the earlier passages in guinea-pigs was put down to insufficient adaptation to this host. For example, Andrews (1931), in discussing procedure, states: "The strains had first to be well adapted to the guinea-pig, and this was in some instances a lengthy process, and at times one of considerable difficulty." In the same report the English Foot-and-Mouth Disease Research Committee (1931), referring to the difficulties experienced in determining the type of certain viruses from the Argentine, say: "When tested on cattle three of them appeared to belong to the type 'O,' though by tests in guinea-pigs the result was not quite so clearly defined, probably because of incomplete adaptation." Again, from the same source, "The only difficulty in using guinea-pigs for this purpose is that it is necessary to make sure that the fresh strain from a bovine is adapted and will readily infect the guinea-pig." In an earlier report of the same committee, Burbury (1928) makes the following statement: "Experience has shown that immunological distinctions in strains found by means of guinea-pigs apply equally to cattle," and later, "In comparing types of virus in guinea-pigs it is important to use only viruses fully adapted to the guinea-pig, so that animals may be equally susceptible to the different strains. Therefore, the first step in typing strains recently derived from cattle has been to adapt the virus to the guinea-pig . . . . This may require from eight to 13 passages."

Through the valuable researches of Trautwein (1927, 1929 and 1932), it was demonstrated that variants of the three types are

frequently encountered, which offer considerable difficulty to rapid typing. Thus in 1927 he refers to an atypical " C " strain which required 94 serial passages in guinea-pigs before it would revert to a pure " C " type, and incidentally mentions that such reversion appears to be favoured by storage in glycerine-buffer mixtures. Trautwein further records that although 100 per cent. of guinea-pigs could be infected successfully with all three standard types, the percentage of cattle that could be so infected was only 72 per cent. for the second and 37 per cent. for the third infection. Moreover, in the same communication (1927) he was able to show that while experimental infection of guinea-pigs with one of the three type strains provoked the formation of strictly homologous antibodies only, similar monovalent infection in cattle brought about the appearance of heterologous antibodies in 52 per cent. of the experimental animals.

In spite of Trautwein's important findings, the belief seems to have steadily gained ground that all bovine viruses in reality belonged to one of the three standard types and that any virus that did not conform to one of the types after a reasonable number of passages in guinea-pigs was to be looked upon as an " aberrant strain " or " variant." Indeed in 1929 we find Trautwein himself commenting with apparent satisfaction on the fact that there was complete agreement between German, English, and French workers to the effect that all strains so far studied in Europe were to be considered as belonging to one of the three standard types. In a later communication (Trautwein and Reppin, 1932) attention was drawn to the existence of " O " variants, capable of infecting guinea-pigs immune to all three standard types, which later by repeated passages in guinea-pigs were induced to revert to the normal " O " form.

The publications of Manninger and Laszlo (1930 and 1932) were the first to introduce an element of doubt into the current assumption that these fixed guinea-pig types could really be considered as representing corresponding fixed types in cattle. Manninger and Laszlo concluded that although considerable immunological differences may exist between bovine strains of the virus, types with fixed characters are not encountered in cattle. The so-called types represent modifications which may overlap or even change into each other, and in nature there are factors that induce a virus to change in the direction from " C " through " A " to the better fixed " O " type. Thus a virus in the course of an outbreak may change its immunological state and acquire the capacity to reinfect animals in which it has already once caused an infection.

How far these conclusions found general acceptance may perhaps be fairly gauged from the comment of the English Foot-and-Mouth Disease Research Committee (1931): " There have been occasional statements such as that of Manninger (1930) that

a strain of one type has changed its character and become assimilated to another type. Such observations are very rare and must at present be accepted with reserve."

It is the object of this paper to draw attention to certain vagaries in the behaviour of several African strains of the virus, with particular reference to their " type " relationship during the earlier passages in guinea-pigs and cattle, which are largely in agreement with the earlier findings of Manninger during his typing of strains recovered from cattle in Austria.

The Kenya Strains of Virus.

A spontaneous outbreak of foot-and-mouth disease at the Research Laboratory, Kabete, in February, 1932, provided the opportunity to make a brief investigation of a few local strains of virus. Already one Kenya strain from the Nanyuki district had been sent home to Dr. Andrews, who classified it as a typical " O " strain. This strain had been recovered from an extensive outbreak of the disease that had caused heavy mortality on many of the affected farms. The Kabete outbreak, by contrast, was relatively benign, and the total mortality only amounted to three head, all of which were young calves.

Three main herds of cattle are kept at the laboratory proper: the dairy herd, the serum-making herd, and the discontinued herd. Infection was discovered in the dairy herd on February 8th, 1932. On the same day a few cases of the disease were also found in the antirinderpest serum-making herd. The disease ran rapidly through all three herds at the laboratory and by February 20th fresh cases had ceased to occur. A careful record was kept of animals affected during the outbreak and relatively few escaped infection. The upper and lower farms attached to the laboratory did not become infected during the 1932 outbreak. Many of the animals had been born at the laboratory, or at one of the two laboratory farms, and since I am assured that neither on the laboratory nor on the farms had there been foot-and-mouth disease during the previous 15 years the susceptibility of the home-bred stock was beyond doubt.

Infections in Guinea-pigs.

On February 8th pooled material from unruptured vesicles on the tongues of cattle was injected into the plantar pads of guinea-pigs, and by the 4th passage the virus was generalising in all the inoculated guinea-pigs. From the 7th passage onwards the virus generalised regularly in 48 hours.

In the meantime " A," " O " and " C " type-strains of virus had been obtained by air mail from Dr. Andrews at Weybridge and infections were set up in guinea-pigs with these strains. Cross-immunity tests were next carried out with the Kabete virus and the standard types, employing guinea-pigs immune to Kabete

virus from the 4th to the 10th passages, and in the converse inoculating guinea-pigs immune to " A," " O " or " C " types with the 11th passage of Kabete virus. The results of these tests are tabulated below.

TABLE I.

1st Infection.	Number of Guinea-pigs.	Result.	2nd Infection.	Number of Guinea-pigs.	Result.
K IV to X	2	2G.	" O "	2	2G.
	7	7G.	" A "	7	2L.5—
	8	8G.	" C "	8	7L.1—
" O "	1	1G.	K XI ...	1	1G.
" A "	3	3G.		3	3—
" C "	2	2G.		2	2—

G signifies generalised infection.
L „ local lesions only.
± „ doubtful local vesicles which receded without rupturing.
— „ no reaction.
† „ a death.

It will be observed that at this stage the Kabete virus protected almost completely against subsequent inoculation with " A " virus, and somewhat less completely against " C "; of the 15 " K " immune guinea-pigs tested with these two strains none showed generalisation. In the converse test both " A " and " C " types protected completely against " K." The virus appeared to overlap these two strains perhaps to a greater extent than in any previously recorded instance (*c.f.* Trautwein, 1927). The result was so striking that a test was promptly set up to verify the independence of the " A " and " C " strains received from Weybridge, and the cross-immunity tests were repeated, this time using guinea-pigs previously infected with the " A " and " C " types from the original Weybridge material. The results of these tests are tabulated (*vide* Table II, p. 263).

Here there is a suggestion that the protection afforded by "C" virus against subsequent infection with Kabete XI is less efficient than that afforded by "A" virus against Kabete, since Kabete after " C " gave almost complete immunity against a third inoculation with " A," whereas Kabete after " A " gave little protection against a third inoculation with " C."

In an attempt to obtain further evidence on this point additional tests were made with the next passage, Kabete XII as the first infection, followed in half the animals first by " A " and then by " C " and in the other half by " C " and finally by " A." The results, also included in Table II, show that KXII gave a definitely greater immunity against " A " than against " C." It is evident from these tests that some transition was in progress, and that the Kabete virus was losing from its antigenic make-up components common with " C."

TABLE II.

1st Infection.	No. of Guinea-pigs.	Result.	2nd Infection.	No. of Guinea-pigs.	Result.	3rd Infection.	No. of Guinea-pigs.	Result.
C I	4	4G.	A II	4	4G.(†)			
C I	2	2G.	K XI	2	2—	A III	2	1±.1—
A I & II	3	3G.	K XI	3	3—	C II	3	1L.2G.
K XII	5	5G.	A III	5	5—	C IV	5	1—.2L.2G.
	5	5G.	C III	5	5L.	A IV	5	5—

TABLE III.

1st Infection.	No. of Guinea-pigs.	Result.	2nd Infection.	No. of Guinea-pigs.	Result.	3rd Infection.	No. of Guinea-pigs.	Result.
C V	4	4G.*	K XXIX	4	1L.3G.	—	—	—
C V	3	3G.*	K XXX	3	1L.2G.	—	—	—
A VI	6	6G.*	K XXIX	6	5—.1L.	C VI	6	2L.4G.
A VI	3	3G.*	K XXX	3	3—	C VI	3	1L.2G.
K XXIV	3	3G.	C VII	3	1—.2G.	—	—	—
K XXIV-XXVII	6	6G.*	A VII	6	5—.1L.	—	—	—
K XXVIII	5	5G.*	A VII	5	5—	—	—	—
C VI	5	5G.*	K XXX	5	5G.	—	—	—
K XXX	6	6G.	O XII	6	6G.(2†)	C V	4	4G.(1†)
O IV	6	6G.	K XXX	4	4G.	C VII	4	4G.

G signifies generalised infection. L signifies local lesions only. ± signifies doubtful local vesicles which receded without rupturing. — signifies no reaction. * indicates that the immunity of the animals was tested with the same type of virus before they were submitted to the second infection. † signifies a death.

Tests of the later passages were made as frequently as possible, but difficulty was experienced because of the increasing virulence of the virus, which brought about the death of most of the inoculated pigs; and attempts to use guinea-pigs vaccinated with formalised virus for these tests had to be abandoned as unsatisfactory. At the 24th passage the Kabete virus failed to protect two out of three guinea-pigs against a subsequent inoculation with " C " virus, but throughout the " K " virus continued to give complete protection against " A." The results of tests made with later passages from the 24th to the 30th are tabulated below. In passages later than the 30th Kabete virus never appeared to have anything in common with " C " virus; in fact it had become a normal " A " strain. (*Vide* Table III.)

A second Kenya virus, received on February 29th, 1932, from an outbreak at Nakuru, roughly 100 miles distant from Kabete, when tested in the earlier passages proved to be immunologically identical with the original Kabete virus, in that it protected against " A " and " C " types, but not quite completely against the latter, and gave no protection at all against " O." Unfortunately, owing to shortage of experimental animals, it was not possible at the time to carry this strain through to final fixation in guinea-pigs, and it failed to survive in cold storage. In its early passages this strain was also incapable of infecting cattle immune to the Kabete strain.

A third local virus was obtained from an outbreak at Mbgathi, about eleven miles from Kabete, in April, 1933. The outbreak in question was said to have originated far south in the Masai reserve, and to have gradually extended northwards to the Nairobi district. This virus, referred to as Cowie, had immunological characters exactly like those of the Kabete virus, as a series of tests carried out with the earlier passage will show (Tables IV and V). Eventually it fixed definitely in guinea-pigs as an " A " type.

Table IV.

1st Infection.	*Number of Guinea-pigs.*	*Result.*	*2nd Infection.*	*Number of Guinea-pigs.*	*Result.*
C.	2	2G.	Cowie XVII	2	1L.1G.
A.	4	2G.	Cowie XVII	4	2±.2—
O.	3	3G.	Cowie XVII	3	3G.
C.	1	1G.	Cowie XXV	1	1G.

G signifies generalised infection.
L „ local lesions only.
± „ doubtful local vesicles which receded without rupturing.
— „ no reaction.

TABLE V.

THE FIXATION OF "COWIE" VIRUS AS AN "A" TYPE.

No. of Animal.	*1st Infection.*	*Number of Guinea-pigs.*	*Result.*	*2nd Infection.*	*Number of Guinea-pigs.*	*Result.*
479 and 480	Cowie II	2	2G.		2	1±.1L.
	„ III	2	2G.		2	1±.1L.
	„ IV	2	2G.		2	1±.1L.
	„ V	2	2G.		2	2±
	„ VI	2	2G.	A	2	1±.1L.
	„ VII	2	2G.		2	2±
	„ VIII	2	2G.		2	2±
	„ IX	2	2G.		2	2±
	„ X	2	2G.		2	2±
	„ XI	2	2G.		2	2—
	„ XII	2	2G.	A	2	2—
	„ XIII	2	2G.		2	2—
	„ XIV	2	2G.		2	2—
	„ XVI to XXXV	16	16G.	A XIII	16	16—

No. of Animal.	*3rd Infection.*	*Number of Guinea-pigs.*	*Results.*	*4th Infection.*	*Number of Guinea-pigs.*	*Result.*
479 and 480		2	2—		2	2G.
		2	1—.1L.		2	2G.
		2	2—		2	2G.
		2	1—.1G.		2	2G.
	C	2	2±	O	2	2G.
		2	1—.1L.		2	2G.
		2	2G.		2	2G.
		2	2±		2	2G.
		2	1L.1G.		2	2G.
		2	1L.1G.1†			
	C	2	2G.2†			
		2	1L.1G.			
		2	1L.1G.1†			
	C	16	16G.7†			

G signifies generalized infection.
L „ local lesions only.
± „ doubtful local vesicles which receded without rupturing.
— „ no reaction.
† „ a death.

THE SOUTHERN RHODESIAN VIRUSES.

THE BELINGWE STRAIN.

On March 21st, 1932, a quantity of virus was received from the Chief Veterinary Surgeon, Southern Rhodesia. The material was pooled glycerinated blood taken from a number of animals naturally infected by contact with inoculated animals at Belingwe, Southern Rhodesia. The outbreak in question was one of the foci remaining from the 1931-2 epizoötic described by Bevan (1932, 1933), and considered by him to present many unusual features, particularly with reference to the low virulence of the virus, its relatively slight contagiousness, and the difficulties

TABLE VI.

THE BELINGWE VIRUS IN ITS EARLY PASSAGES AS AN "O" TYPE.

1st Infection.	No. of Guinea-pigs.	Result.	2nd Infection.	No. of Guinea-pigs.	Result.	3rd Infection.	No. of Guinea-pigs.	Result.	4th Infection.	No. of Guinea-pigs.	Result.
K VI	2	2G.	C II	2	2L.	B IV	2	2G.	—	—	—
K IX	2	2G.	C II	2	2L.	B IV	2	2G.	—	—	—
K VIII	3	3G.	A III	3	2—.1L.	B VII	3	3G.	—	—	—
A III	2	2G.	B VII	2	2G.	—	—	—	—	—	—
C I	4	4G.	A II	4	4G.(1†)	B VII	3	3G.	—	—	—
O I & II	2	2G.	B IX	2	2—	—	—	—	—	—	—
K VI-IX	3	3G.	O II	3	3G.	C II	3	2—.1G.	B VII	3	2—.1G.
K VI-IX	2	2G.	C II	2	2—	O III	2	2G.	B IX	2	2—.
O I	2	2G.	C II	2	2G.(1†)	B VII	1	1—	—	—	—
K VII	2	2G.	O III	2	2G.	B IX	2	2—	—	—	—

G signifies generalised infection. L signifies local lesions only. — signifies no reaction. † signifies a death.

TABLE VIII.

BELINGWE XVIII-LIII. OVERLAP WITH "A" AND "C."

1st Infection.	No. of Guinea-pigs.	Result.	2nd Infection.	No. of Guinea-pigs.	Result.	3rd Infection.	No. of Guinea-pigs.	Result.
B XVIII (stored)	4	4G.	B XIII (stored)	4	4—	A VIII	4	4—
A VI	3	3G.*	B XXVIII	3	3L.	—	—	—
A XIII	4	4G.	B XXVIII	4	4—	—	—	—
B XVIII	2	2G.	B XXIII	2	2—	A XIII	2	2—
A VI (stored)	3	3G.	B XXVII	2	2±	—	—	—
B XXIII	5	5G.	C XII	5	3L.2G.	—	—	—
A XV	2	2G.	B LIII	2	2—	—	—	—
C XIII	2	2G.	B LIII	2	2±	—	—	—

G signifies generalised infection. L signifies local lesions only. ± signifies doubtful local vesicles which receded without rupturing. — signifies no reaction. * indicates that the immunity of the animals was tested with the same type of virus before they were submitted to the second infection.

experienced in setting up infection in cattle and guinea-pigs by normal methods.

Infection of Cattle.

Glycerinated blood received from Mr. G. C. Hooper-Sharpe, District Veterinary Surgeon, Bulawayo, was inoculated intramuscularly in a dose of 5 c.c. into two cattle, X7884 and X7899. In order to ensure infection, the virus was also squirted into the nasal passages after scarification of the mucous membrane, and at the same time the dental pad was scarified and virus was rubbed into the membrane. On the 6th day after the inoculation X7884 showed a number of small vesicles on the dorsal surface of the tongue. This reaction was preceded by an evening temperature of 104·6°. X7899 developed extensive vesicles on the tongue on the 9th day, without ever having shown a temperature reaction. Two other susceptible cattle, X7882 and X7888, were inoculated intranasally with scarification, using the original blood. These two cattle showed definite unruptured vesicles on the 12th day after inoculation. These vesicles appeared simultaneously with those in a contact animal, X3009, which had been kept in the same loose-box as the four inoculated cattle.

Two cattle immune to the Kabete virus were also inoculated intramuscularly and by scarification of the dental pad with the original material from Bulawayo. Both these animals developed typical lesions on the 6th day. With material collected from the vesicles in X7884, two additional cattle, immune to " K " virus, were inoculated by scarification. Both animals contracted a typical infection. Four heifers, bred on the laboratory farm and presumably susceptible to the Kabete virus, which had been boarded out at one of the Veterinary Training Depôts for a period of eleven months, were also inoculated intramuscularly and by scarification with the original material. None of these four animals reacted.

One can conclude then, that on arrival the first Rhodesian or Belingwe virus, as it will be called, was not identical in cattle with " K " virus. (*Vide* Table IX.)

Infection of Guinea-pigs.

The first attempt to produce infection in guinea-pigs with the blood received from Bulawayo was successful, and all the guinea-pigs inoculated developed local lesions. Two animals out of eight inoculated with the original material showed generalisation. The virus was particularly easy to adapt to guinea-pigs, and by the 5th passage generalisation was taking place regularly within 48 hours. From the 4th passage onwards there was mortality among the guinea-pigs. As in the previous experiments with the Kabete strain of virus, an effort was made to carry out immunity tests with the earlier passages of the Belingwe strain. The results of these earlier tests are tabulated (*vide* Table VI).

It will be observed that the Belingwe virus IV to IX was able to infect animals that had previously been inoculated with " A "; " C "; " K " and " C "; " K " and " A "; " K," " C " and " A "; but that with one exception out of ten animals inoculated it failed to infect guinea-pigs that had previously been infected with " O "; " O " and " C "; " O," " K " and " C "; " O " and " K."

In later passages two guinea-pigs, previously infected with " O," both showed a local lesion only when inoculated with BXX, and one guinea-pig, immune to Belingwe VII, showed no resistance to infection with BXX, which produced a second generalisation. Guinea-pig 139, immune to Belingwe IX, and guinea-pig 149, immune to Belingwe XVII, both developed local lesions when inoculated with BXX. Five guinea-pigs, two immune to XV passage of Belingwe virus, and three immune to passage XVII all reacted to an inoculation with " O " virus, generalisation taking place in 24 hours. The reactions were peculiar in that the local lesions receded about the time the forefeet and mouth lesions made their appearance. Of the five animals, the local lesions in two only contained any demonstrable quantity of lymph. At the 22nd passage the Belingwe virus infected two pigs immune to " O " virus, producing generalised lesions in one and local lesions in the other. At the 23rd passage this strain infected four guinea-pigs immune to " O " virus, producing generalised lesions in each of them and killing one out of the four.

TABLE VII.

Belingwe Virus. The Loss of " O " Components During Passages IX to XXV.

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection.*	*No. of Guinea-pigs.*	*Result.*
O III	2	2G.*	B XX	2	2L.
B VII	1	1G.		1	1G.
B IX	1	1G.		1	1L.
B XVII	1	1G.		1	1L.
O III	2	2G.	B XXII	2	1L.1G.
B XV-XVII	5	5G.	O IX	5	5G.‡
B XX	3	3G.*	O XI	3	3G.
B XX-XXV	5	5G.	O XII (stored)	5	5G.(2†)
O VII	4	4G.	B XXIII	4	4G.(1†)

G signifies generalized infection.
L „ local lesions only.
* „ tested.
† „ a death.
‡ See remarks in text.

In the converse five guinea-pigs, immune to the Belingwe virus between the 20th and the 25th passages, showed normal generalisation on inoculation with " O " virus, and two died.

A test was next made of the immunity of four guinea-pigs inoculated with BXVIII, which had been stored for four months, against BXIII, which had been stored for seven months. No infections resulted. The same guinea-pigs were then tested by an inoculation of " A " virus, which also failed to take. (*Vide* Table VIII.)

Three " A " immunes were then tested with " A " virus and afterwards inoculated with BXXVIII. All three developed local lesions only to the inoculation of " B " virus.

Four " A " immunes tested with BXXVIII failed to react at all to the inoculation.

Two "B" immunes that had reacted severely to BXVIII, and had been tested with BXXIII with a negative result, failed to react when inoculated with " A " virus.

Later BXXVII produced local lesions that receded without rupturing in three guinea-pigs immune to " A " virus.

Of five guinea-pigs immune to BXXIII and tested with BXXIV that were inoculated with " C " virus, two reacted with generalisation and the remaining three showed local lesions only.

At this stage there was a good deal of similarity between " B " virus and the Kabete strain ; and a further series of rapid passages in guinea-pigs, bringing the total number of passages up to 53, failed to fix this strain as one definite type. It was still possible to protect against BLIII by previous infection with either " A " or " C " virus. The examination of this strain will be continued at a later date.

THE MSASE VIRUS.

A second virus was received from Mr. Hooper-Sharpe on April 8th, 1932. This virus was collected at the Msase Mission, which is between 35 and 40 miles from High Peak Farm, Belingwe, where the first of the two Rhodesian viruses was obtained. The cattle at Msase Mission had acquired infection from other naturally infected animals in the adjacent reserve.

Infection of Cattle.

Ten cattle were inoculated intramuscularly with this virus on the day it arrived. Four of them were immune to the Kabete virus and six completely susceptible. In addition, four completely susceptible animals were placed in contact with the inoculated ones after each of them had received an injection of 200 c.c. of polyvalent foot-and-mouth serum from the Riems station. Of the animals immune to the Kabete virus, two reacted and two failed to react. Of the remaining six animals all reacted as shown in the table below. Of the four contact animals, exposed during the reaction of the inoculated animals, all reacted in spite of the protective inoculation of serum. In a further test of the capacity of this virus to infect animals immune to the Kabete virus, six Kabete immune animals were inoculated with material from X5950 above and five

out of the six reacted. Part of the same material was inoculated into four animals immune to the Belingwe virus as the result of recent infections, and into two animals immune to both Belingwe virus and Kabete virus. Of the four Belingwe immunes, one, No. 7884, showed two definite lesions on the tongue on the 5th day; another, No. 7888, gave a temperature reaction on the 9th and 10th days, but did not develop lesions. Neither of the Belingwe and Kabete immunes reacted.

A passage of the Msase virus from No. X6823 was then inoculated into two Kabete and Belingwe immunes and into the four boarded-out heifers that had once previously failed to react to an inoculation of the first Belingwe virus. Of the two Kabete and Belingwe immunes one, No. 5435, reacted definitely, showing a small vesicle at the back of the tongue on the 7th day, and the other was negative. One out of the four heifers that had resisted inoculation with the Belingwe virus reacted to the Msase virus, while the other three were refractory. (*Vide* Tables IX and X.)

Six additional animals, two completely susceptible, two " K " immunes and two " K " and " B " immunes, were also inoculated with the passage of this virus from X5950, and all reacted except 5918, one of the " K " and " B " immunes. The five recovered animals were inoculated with " O " virus; roughly four months later; all of them reacted to this inoculation, and all proved immune to a further test inoculation with " O " virus one month later.

At the time of arrival at the laboratory the Msase virus was able to infect eight out of 14 animals immune to the Kabete virus, and to break down the immunity of three out of twelve animals immune to the Belingwe virus. Two of these three cattle had been definitely infected with " B " virus only a few weeks previously. There is evidence in these tables also that infection with " M " virus at that time did not afford any immunity against subsequent infection with " O " virus.

Infections in Guinea-pigs.

The Msase virus was successfully passaged at the first attempt in guinea-pigs and on the 3rd passage generalised. In passages from the 6th to the 10th there was a tendency for the virus to die out, and it was necessary once at the 10th passage to transfer lymph from vesicles on the forefeet owing to failure of the inoculations with lymph taken from the initial lesions.

Very few immune guinea-pigs were available for tests with the earlier passages, but the record of the reactions in guinea-pigs 43 and 44 demonstrates that guinea-pigs immune to " A," " K " and " C " were infected with MVI with generalisation, and that subsequently these animals reacted to "O" virus. (*Vide* Table XII.)

MIX produced a generalised infection in two guinea-pigs that had passed through an " O " infection seven weeks previously and

whose immunity had been confirmed by a test. At the 11th passage " M " virus infected four guinea-pigs immune to the Belingwe virus. The disease generalised in all the animals. At the 13th passage a further four Belingwe immunes were infected with generalisation. These animals had recovered from the infection with Belingwe passages from the 11th to the 14th. Three months later four surviving guinea-pigs from this test were inoculated with " O " virus and all of them resisted infection. (*Vide* Table XIII.)

At the 19th passage " M " virus infected four guinea-pigs immune to " A " and " C," producing generalisation in them all. At the 15th, 16th, 18th, 21st and 26th passages it failed to infect guinea-pigs immune to " O " virus. (*Vide* Tables XIV and XV.)

Infection was then set up with stored material from the 14th passage. After a further two passages this stored virus was inoculated into two " O " immunes and to " A," " C," and " O " immunes. No infection resulted.

The next passage of this stored virus failed to infect four " O " immunes and two " O " and Belingwe immunes, but successfully infected five Belingwe immunes and two "C" and "A" immunes.

These tests indicate that while from the 5th to the 10th passage " M " virus afforded no immunity against " O " virus, by the 15th passage complete protection was obtained in either direction. From the 19th passage onwards the direct strain of " M " virus behaved in guinea-pigs as a normal " O." This was equally true of the second strain of " M " virus recovered from the stored MXIV material after three months.

Infection of Cattle with Guinea-pig Strain.

At the 22nd guinea-pig passage the current Msase virus was taken back to cattle, and cross-immunity tests with " O " virus were carried out in both directions. Of ten animals employed in these cross-immunity tests nine were definitely protected by the first infection. One animal, X8764, failed to react to an inoculation with " O " virus, although it showed a febrile reaction on the 6th day, which may or may not have been due to infection. When this animal was inoculated with MXXII it was the only animal out of four in the experiment to contract infection. On the whole the evidence in favour of the identity of Msase XXII onwards and the standard "O " type may be regarded as pretty conclusive. (*Vide* Table XI.)

STORED MSASE VIRUS FROM PASSAGE IV.

Material from an earlier passage, MIV, stored at a time when the " M " virus was certainly not identical with " A," *i.e.*, at the stage when it was capable of provoking infections in animals immune to " A," " O " and " C," was taken from cold store after five months. It was at once realised that this strain was no

TABLE IX.

THE BELINGWE VIRUS IN CATTLE: CROSS-IMMUNITY TESTS WITH KABETE AND MSASE.

No. of Animal.	*1st Infection.*	*Route.*	*Reaction.*	*2nd Infection.*	*Route.*	*Reaction.*	*3rd Infection*	*Route.*	*Reaction.*
7884	Belingwe I blood 21/3/32	Intramuscular & Intranasal & Pad scarification	Temp. 5th day, extensive eruption tongue & palate	Msase II from X5950 6/5/32	Intramuscular	2 small vesicles tongue 5th day	—	—	—
7899	„	„	Vesicles tongue 9th day	„	„	Negative	—	—	—
7882	„	Intranasal	Temp. 9th day, vesicles tongue 12th day	„	„	Negative	—	—	—
7888	„	„	Temp. 9th day, vesicles 12th day	„	„	Temp. 9th & 10th days No lesions	—	—	—
5260	Kabete virus	Natural infection	Extensive lesions	Belingwe II from 7884 24/3/32	Scarification pad	Extensive lesions tongue 6th day	Msase II from X5950 6/5/32	Intramuscular	Negative
6002	„	„	„	„	„	Vesicles tongue 9th day	„	„	„
5918	„	„	„	Belingwe I blood 21/3/32	Intramuscular, Intranasal & Pad scarification	Vesicles tongue 6th day	Msase III from 6823 25/4/32	„	Negative
5435	„	„	„	„	„	Vesicles tongue 6th day	„	„	Vesicles back of tongue 7th day‖
§5473	Belingwe I blood 21 3/32	Intramuscular & scarification	Negative	Msase III from 6823 25/4/32	Intramuscular	Negative	—	—	—
§5447	„	„	„	„		Negative	—	—	—
§5471	„	„	„	„	„	1 vesicle tongue 5th day	—	—	—
§5438	„	„	„	„	„	Temp. 3rd No lesions			

§ The four animals marked § were heifers that had been born at the laboratory farm, but which had been boarded out for a period of 11 months at one of the Veterinary Training Centres. ‖ This animal, Number X5435, was 3½ months later successfully infected with "O" virus, and 5 weeks later tested again with "O" virus with a completely negative result.

TABLE X.

MSASE VIRUS: CROSS-IMMUNITY TESTS WITH KABETE AND "O" VIRUSES.

No. of Animal.	*1st Infection.*	*Route.*	*Reaction.*	*2nd Infection.*	*Route.*	*Reaction.*
6829	Kabete virus	Natural infection	Extensive lesions	Msase I blood 20/4/32	Intramuscular	Negative
7731	,,	,,	,,	,,	,,	,,
7598	,,	,,	,,	,,	,,	Temp. 6th day, lesions 13th ,,
6823	,,	,,	,,			Temp. 5th day, extensive lesions 5th day
7005	Msase I blood 20/3/32	Intramuscular	Temp. 3rd day, lesions 4th day	—	—	—
7207	,, ,,	,,	Temp. 6th day, lesions 8th day	—	—	—
6574	,, ,,	,,	Temp. 7th day, lesions 10th day	—	—	—
7270	,, ,,	,,	Temp. 3rd day* lesions 4th day	O IX 15/8/32	Intralingual	Temp. and extensive lesions 24 hours
7006	,, ,,	,,	Temp. 2nd day,* lesions 4th day	O IX 15/8/32	,,	,,
7352	,, ,,	,,	Temp. 4th day* lesions 4th day	O IX 15/8/32	,,	,,
7359	Contact with 6 animals 7005 to 7352 Msase I Each received 200 c.c. of Reims polyvalent Foot and mouth hyperimmunent serum	,,	Temp. 3rd day, lesions 4thday	—	—	—
6850		,,	Temp. 3rd day, lesions 4th day	—	—	—
5950		,,	Temp. 7th day, lesions 10th day	—	—	—
6808			Temp. 6th day, lesions 6th day	—	—	—
6025	Kabete virus and convalescent serum 4/3/32	Intramuscular	Lesions 4th day	Msase II from 5950 6/5/32	Intramuscular	Temp. 3rd day, lesions 3rd ,,
6881	,, ,,	,,	Lesions 4th day	,,	,,	Negative
6295	,, ,,	,,	Temp. 3rd day, lesions 5th day	,,	,,	Temp. & lesions 3rd day
7536	Kabete virus and hyperimmune serum	,,	Lesions 8th day	,,	,,	Small lesion 5th day
7054	,, ,,	,,	Temp. 5th day	,,	,,	Temp. & lesions 3rd day
7553	,, ,,	,,	Lesions 4th day	,,	,,	Temp. 3rd days doubtful lesion, 13th day

* Immunity to Msase tested by intralingual inoculation before infection with "O" virus.

TABLE XI.

CROSS-IMMUNITY TESTS WITH THE GUINEA-PIG STRAIN OF MSASE FIXED BY DIRECT PASSAGE AS AN "O," THE GLYCERIN-BUFFER STRAIN FIXED AS "A," AND "O" VIRUS.

No. of Animal.	*1st Infection.*	*Result.*	*2nd Infection.*	*Result.*	*3rd Infection.*	*Result.*
*8773	"O"	Temp. reaction + vesicles before 3rd day	MXXII (direct strain)	No reaction		
*8770		Temp. reaction + vesicles tongue 3rd day		No reaction		
*8764		Temp. No lesions 6th day		1 small vesicle on tongue 5th day		
*8758		1 vesicle on tongue, ruptured 9th day		No reaction		
*8772	MXXII (direct strain)	Vesicles tongue 2nd day, temp. 3rd and 4th days	"O"	No reaction	M XIX (stored strain 14 mths.)	Vesicles tongue and pad 3rd day
*8762		Vesicles tongue 2nd day, temp. 3rd and 4th days		No reaction		Temperature and vesicles 2nd day
*8600	"O"	Lesions 5th day	MXXVII (direct strain)	No reaction		Temp. and lesions 3rd day
*9014		Lesions tongue 4th day		No reaction		Lesions and temp. 2nd day
8658	MXXVIII (direct strain)	Lesions and temp. reaction 2nd day	MXXVII (direct strain)	No reaction		Temp. and lesions 3rd day
8273		Temp. 2nd and 3rd days, lesions 3rd day		No reaction		" "
8876		Lesions 3rd day		No reaction		" "
8702		Lesions 3rd day		No reaction		Temp. and lesions 2nd day
8700		Lesions 3rd day		No reaction		Temp. and lesions 3rd day
8697		Lesions 3rd day		No reaction		Temp. and lesions 5th day
*9021	"O"	Temp. 1st day, lesions 2nd day	MXX (direct strain)	No reaction		Temp. and lesions 3rd day
*9037		Temp. 1st day, lesions 2nd day		No reaction		High temp. and lesions 2nd day

* Immunity to Msase or "O" virus tested by intralingual inoculation before the second infection.

TABLE XII.

MSASE VI AS AN INDEPENDENT TYPE.

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection.*	*No. of Guinea-pigs.*	*Result.*	*3rd Infection.*	*No. of Guinea-pigs.*	*Result.*	*4th Infection.*	*No. of Guinea-pigs.*	*Result.*	*5th Infection.*	*No. of Guinea-pigs.*	*Result.*
A III	2	2G.	K XI	2	2—	C I	2	1L.1G.	M VI	2	2G.	O III	2	2G.

G signifies generalised infection. L signifies local lesions only. — signifies no reaction.

TABLE XIII.

MSASE VI TO XII, DISTINCT FROM "O" AND BELINGWE (EARLY PASSAGES).

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection.*	*No. of Guinea-pigs.*	*Result.*	*3rd Infection.*	*No. of Guinea-pigs.*	*Result.*
O III	2	2G.*	M IX	2	2G.	—	—	—
M VI-VII	3	3G.	O II (stored)	3	3G.(1†)	B XX	2	2
B XI-XIV	4	4G.	M XI	4	4G.(1†)	O IX	2	2—
	4	4G.	M XII	4	4G.(1†)		2	2—

G signifies generalised infection. L signifies local lesions only. — signifies no reaction. * indicates that the immunity of the animals was tested with the same type virus before they were submitted to the second infection. † signifies a death.

TABLE XIV.

MSASE XV-XXVI FIXATION AS AN "O" TYPE.

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection.*	*No. of Guinea-pigs.*	*Result.*	*3rd Infection.*	*No. of Guinea-pigs.*	*Result.*
O IV	2	2G.	M XV	2	2—	B XX	2	1L.1G.
O IV	2	2G.	B XX	2	2L.	M XV	2	2—
O VIII	4	4G.	M XVI	4	4—	B XXIX	4	4G.
O VI	5	5G.	M XVIII	5	5—	B XXIII	5	5G.
M XV	2	2G.	O XII	2	2—	B XXIII	2	2G.
M VI	1	1G.	M XV	1	1G.	B XXIII	1	1—
M XII	2	2G.	M XV	2	2±	B XXIII	2	2G.
O III	2	2G.	M XXVI	2	2—	—	—	—

G signifies generalised infection. L signifies local lesions only. ± signifies doubtful local vesicles which receded without rupturing. — signifies no reaction.

TABLE XVI.

MSASE IV STORED IN GLYCERIN 4 MONTHS: AN "A" TYPE.

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection*	*No. of Guinea-pigs.*	*Result.*	*3rd Infection.*	*No. of Guinea-pigs.*	*Result.*
M IV (stored)	4	4G.	M XX (current)	4	4G.(2†)	C II	2	2G.
M XVIII and XX (current)	2	2G.	M V (stored)	2	2G.	C II	2	2G.
M V and VI (stored)	2	2G.	A III	2	2—	C III	2	2G.
M VI and VII (stored)	2	2G.	A X	2	2—	O XIV	2	2G.

G signifies generalised infection. L signifies local lesions only. — signifies no reaction. † signifies a death.

longer immunologically identical with the current strain of " M," as the tabulated inoculations will show. Cross-infection was possible between the two strains in either direction. Further tests indicated that this stored " M " virus had become transformed into " A " (*vide* Table XVI).

Ten months later the remainder of the MV virus was withdrawn from cold store. The results of the inoculations with this strain are shown in Table XVII. The original stored material did not give complete immunity against " A " or against BXXVIII, but a series of rapid passages fully restored its power to protect against " A."

TABLE XV.

Msase XV-XXI as a Pure " O " Type.

History.	*Test Virvs*	*No. of Guinea-ptgs.*	*Result.*
A and C Immunes	M XIX	4	4G.
O and B Immunes	M XXI	3	3—
O Immunes	M XXI	3	3—
O Immunes	M XVI (stored)	2	2—
A C O Immunes	M XVI (stored)	2	2—
O Immunes	M XVII (stored)	4	4—
O and B XXV Immunes	M XVII (stored)	2	2—
B XXVI Immunes	M XVII (stored)	5	5G.
C & A Immunes	M XVII (stored)	2	2G.
M XV-XX Immunes	O XIII	5	5—
O X-XII Immunes	M XIX	5	5—

G signifies generalized infection.
L „ local lesions only.
— „ no reaction.

TABLE XVII.

Msase IV Stored in Glycerin 14 Months, a Weak " A " Type, Restored by Rapid Passage.

1st Infection.	*No. of Guinea-pigs.*	*Result.*	*2nd Infection.*	*No. of Guinea-pigs.*	*Result.*
MIV (original stored material)	4	4G. (slow, 1†)	A XIII	3	2L.1G.
	3	3G.	B XXVIII	3	1L.2G.
M X-XXIX (rapid passages)	13	13G.	O V	13	13G.(6†)
	4	4G.	C IV	4	4G.
	6	6G.	A XIV	6	6—

G signifies generalized infection.
L „ local lesions only.
— „ no reaction.
† „ a death.

Stored Msase Virus in Cattle.

In Table XI will be found the results of inoculating the 19th passage of the " Stored " or " A " strain of Msase virus into cattle that had recently passed through infections with either the current or regularly passaged guinea-pig strain of Msase or the standard " O " virus. All the cattle used were first tested on their immunity to the " current " strain or to " O " virus before the test with " stored " virus was made. When infected by the intramuscular route with MXIX, " stored," every one of twelve cattle reacted definitely to the inoculation, thus confirming the results of previous tests made in guinea-pigs.

Discussion and Summary.

Of the Kenya viruses dealt with in this paper, the Kabete strain when first transferred from cattle to guinea-pigs almost completely overlapped both " A " and " C " types. As a result of direct passage this strain fixed in guinea-pigs as an " A." The Nakuru virus obtained a few weeks later appeared to be similar, while the Cowie virus collected a year later in the same district was again in its earlier passages a combination of " A " and " C."

From the results of the comparison between the Kabete and the Cowie strain, one can conclude that this power to overlap " A " and " C " types had in all probability been maintained unchanged in cattle during the period of more than twelve months; and that the behaviour of these strains in the earlier guinea-pig passages does not justify their characterisation as " aberrant forms " or " variants," but that the particular type should be accepted as a relatively stable one in cattle.

The Belingwe virus started in guinea-pigs as an " O " type; in cattle at this stage it was able to infect animals immune to the Kabete strain, *i.e.*, to " A " and " C," and it is noteworthy that in the one instance where a test was made an animal immune to Kabete and Belingwe was later infected with " O " virus.

The data included in Tables VI, VII and VIII, meagre as they are, indicate that the change in the Belingwe strain from the " O " to the " A "+" C " type, or " A " variant as it would usually be termed, was a gradual one, and from Table VIII it is evident that after this transition the Belingwe strain overlapped both "A" and " C " types much in the same way as the Kenya virus had done during its earlier passages in guinea-pigs. However, an additional series of 25 rapid passages of the Belingwe virus failed to bring about the definite fixation of this strain as a pure " A."

The second Rhodesian virus, Msase, was from the outset peculiar; in that in its first passage in cattle it infected not only Kabete immune animals, but also three Belingwe immunes, two of which had actually been infected with Belingwe virus less than one month previously. In these earlier passages in cattle the

Msase virus did not confer any immunity against subsequent infection with " O " virus.

In response to a question as to the origin of these two Rhodesian viruses, Mr. Hooper-Sharpe stated : " There is not the slightest doubt that the infection at Msase Mission was an extension from the outbreak in the north of the Belingwe reserve from which the Belingwe virus was obtained." The two outbreaks were roughly 35 to 40 miles apart. According to Mr. Hooper-Sharpe the one difference between the two strains was that the Belingwe virus was taken from animals that had contracted the disease by contact with inoculated cattle, whereas the Msase virus was taken from animals that had contracted the disease from contact with other animals naturally infected in the reserve.

During its earlier passages in guinea-pigs the behaviour of the Msase virus was an exact repetition of its behaviour in cattle at Kabete. It was able to infect guinea-pigs immune to " A " and " C " types, and afterwards these animals could be infected with " O." Up to the 9th guinea-pig passage, it retained its power to infect " O " immunes and early " B " immunes, but by the 15th passage it had become an " O " type, and so it remained for more than 30 passages. Taken back to cattle at the 17th direct guinea-pig passage, it was found that it had lost its independence from the " O " type. In tests carried out with the later passages there could be no doubting its identity with the " O " type in cattle.

Virus taken in the form of stripped pads at the 4th guinea-pig passage and stored for periods of 5 or 14 months was found on the resumption of guinea-pig passages to have become fixed as an " A " type. This " stored " strain could not be induced by rapid serial passages in guinea-pigs to mutate further. At the time these pads were stored this virus was certainly not an ordinary " A " type, witness the behaviour of MVI in guinea-pigs 43 and 44 quoted above, and the behaviour of the original virus in Kabete immune cattle.

In connection with the mutation undergone by the virus during cold storage in glycerine-buffer solution, attention is again drawn the Trautwein's statement, quoted in the introduction to this paper, that reversion to a fixed type is favoured by cold storage in glycerine-buffer solution. This view has also received support from Manninger and Laszlo.

It is reasonable to conclude that the ordinary method of typing viruses in guinea-pigs amounts in reality to fixation for a host not naturally susceptible, and that this process of fixation involves the suppression of certain of the antigenic components of the virus, if one may borrow the analogy from bacteriological terminology. These suppressed components may be restored by further passage in cattle, *c.f.* Trautwein's finding that guinea-pig fixed types provoked the formation of heterologous antibodies in 52 per cent. of inoculated cattle.

Normally, no doubt, the state of a virus in cattle is such that, while the virus should probably be considered as polyvalent, one type component is predominant, and fixation in the guinea-pig results in suppression of the weaker factors. Again, to make use of a bacteriological analogy, the cattle viruses might be compared to the non-specific phase and the guinea-pig fixed types to the specific phase of the diphasic group of *Salmonella*, though the analogy is not a very close one. But the history of the Msase strain indicates that fixation does not always take the same direction, and therefore that the type eventually fixed in guinea-pigs does not necessarily indicate the component that was predominant when the virus was last spreading naturally in cattle.

No doubt the " O " type is the commonest of the types in cattle, as indicated both by the number of times it has been recovered through guinea-pig fixation, and by its demonstration through direct tests carried out on immune cattle. It is possibly the most stable of the types in cattle, as concluded by Manninger, but even the " O " type must be considered as liable to antigenic variation, if one accepts the evidence given in this paper concerning the Rhodesian viruses.

The work carried out in recent years on the guinea-pig types has been of great value in drawing attention to the need that antisera should be polyvalent. It has, moreover, enabled workers to establish the fact that immunity against one type of virus is much more durable in cattle that was at first supposed. It has still more recently revealed the existence of bovine strains which antigenically are not covered by the existing three types; and it has led finally to the conception, not yet generally accepted, of the bovine virus as a labile organism liable to spontaneous phase variations so great that they may make possible the re-infection of cattle through which the virus has quite recently passed. Evidence has been presented to show that repeated passage through the guinea-pig may be responsible for changes in the type of a virus; it seems probable that the natural passage of a virus through species other than cattle, *viz.*, sheep, pigs, and wild ruminants, may prove to be an important factor in the production of type-variations during the course of an epizoötic in cattle.

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A STUDY ON "*CELL-INCLUSION DISEASE*" IN FOWLS

I. On the Identity of *Acute Cell-Inclusion Disease* and *Fowl-Plague*

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A disease of fowls characterised by chromatic "inclusions" in the leucocytes was first described by Macfie[1] as occurring in an acute form among fowls in Nigeria. In 1925 Adler[2] reported the existence of what appeared to be an identical, but chronic, form of the disease among fowls in Palestine. In 1931 Gilbert *et al.*[3] made an extensive study of this condition, and they concluded that "a definite disease, which for want of a better name was known as *Cell-Inclusion* or *Macfie's Disease,* occurred among fowls in Palestine." Various forms of the disease have been described: (*a*) *Acute Cell-Inclusion Disease,* a severe and fatal form, in which death usually took place between the second and fifth day after inoculation; (*b*) *Chronic form,* which may be severe or mild, but is not usually fatal.

Recently a severe epizoötic outbreak occurred among fowls at Petach-Tikva. This disease has been diagnosed and described by Gilbert[4] as *Acute Cell-Inclusion Disease.* The nature of the outbreak and the *post-mortem* lesions were suggestive of *Fowl-Plague.* A strain of the virus was, therefore, secured and investigations carried out, to ascertain whether or not *Acute Cell-Inclusion Disease* was identical with *Fowl-Plague.*

This paper deals with the results of these investigations.

History of the Outbreak.

On 16/3/34 a poultry instructor working in the vicinity drew our attention to a disease raging among fowls in and around the township of Petach-Tikva which, according to the information obtained from the local veterinary officer, had broken out about three weeks previously. At the onset the disease had been diagnosed as *Fowl Cholera,* and *Fowl Cholera Immune Serum* ("Bayer") had, it was said, been used with a certain measure of success. Consequently poultrymen in the vicinity insisted on vaccinating all their healthy flocks against *P. avicida.* All known infected flocks in Petach-Tikva have been visited by the author and in every case the mortality proved to be 100 per cent. However, on several farms pigeons associated with affected fowls had not succumbed to the infection. According to the owners, some of the birds showed no symptoms, went to roost healthy and were found dead in the morning; whereas others exhibited convulsions

just prior to death. Most of the owners stated that they could recognise the doomed bird, for it laid a soft egg a day prior to death.

On Mr. F.'s farm all birds died except one; it was found standing under the bushes with eyelids closed, comb and wattle somewhat darkened, and occasionally the bird passed soft greenish droppings. On closer examination the wattles and ear lobes were found to be swollen. This bird, together with several other live and dead birds from various farms, was taken to the laboratory for examination. None of the live birds survived more than 48 hours. The dead birds were examined, *post-mortem* changes noted, and cultures made from the heart blood and liver. All cultures except one remained negative. A 1 : 10 emulsion of liver and spleen in normal saline has been prepared from natural cases, and chickens and pigeons inoculated i.m. One inoculated pigeon died within 24 hours, and the rest remained unaffected. Six subsequently inoculated pigeons also did not succumb. The inoculated chickens died on the third or fourth day.

A mixed liver and spleen emulsion in normal saline was prepared from a natural case and filtered through a Seitz filter. The bird receiving the filtrate died on the fifth day.

Epidemiology.

Practically in every case investigated the disease broke out following the introduction of live or dead birds into the flock, and in one case the infection was apparently due to infected crates. The following outbreaks may serve as examples.

(I) A flock of *native* birds into which newly purchased birds were introduced was completely annihilated, but the adjacent farm of S.C.W.L., separated from the first only by a chicken wire fence, did not suffer any loss.

(II) Mr. B. E.'s flock was ranging in his orange grove. A friend asked him for permission to place a net in the grove to trap a jackal. As the jackal was not caught it was then suggested that the net should be baited with a dead bird; following the introduction of the dead bird Mr. B. E.'s fowls started to die, and all perished in about a week.

(III) A poultry dealer who suffered a severe loss as a result of the disease resolved to purchase birds from premises known to be clean. Two crates of birds were sent to him, and eventually transferred to his own crates. All the birds succumbed. The farm from which the birds originated did not suffer any loss.

The disease soon made its appearance in other districts, and on 29/4/34 was discovered in the Jerusalem market. In this district mostly poultry dealers and backyard poultry raisers sustained heavy loss. In one establishment in which over 200 chickens were kept all died, while two old geese and young and old pigeons remained alive.

Experimental Work.

(I) *Period of Survival.*

(1) Out of the first 47 birds inoculated intramuscularly with the *Petach-Tikva Virus* (P.T.V.), 14 were found dead on the second day, 19 on the third day, 13 survived less than four days, and one bird died on the fifth day .

(2) The virus obtained from a field case was passaged seven times in series. As each inoculated bird died the liver and spleen were removed under aseptic precautions, and 1 c.c. of a 1 : 10 emulsion from those organs in normal saline inoculated i.m. The first bird died on the fourth day, the second and third birds survived two days, and the fourth bird was found dead on the second day. No further increase in virulence was noted.

It appears from the above experiments that the P.T.V. differs from the *Atypical strain of Fowl Plague* isolated by Lagrange[5] in Egypt in the period of survival, which according to Purchase[6] is four to five days with a maximum of nine days. The period of survival with the P.T.V. appears to be identical with that of typical *Fowl-Plague,* which according to Purchase[6] is one-and-a-half to two-and-a-half days, with a maximum of four days.

(II) *Symptoms.*

In a few cases, birds inoculated with the P.T.V. died without showing any symptoms. In most cases, however, the infected bird was depressed, appetite impaired, feathers ruffled, eyelids closed, and the head hidden under the wings. The conjunctiva was usually reddened, and the comb covered with purple blotches. Such symptoms are common to a number of diseases of poultry. Some of the birds, however, showed peculiar changes which are described below and which appear to be of diagnostic importance.

(1) *Swelling of the Head Parts.*—In the early passages of the virus many of the birds showed swelling of the head parts. The wattles were the most common seat of the swelling; the ear lobes and eyelids followed in frequency. The degree of swelling varied from one noted only on palpation to a distinct visible swelling. The swelling was usually bilateral, but occasionally it was unilateral. When the swollen parts were cut into, the tissues were found to be infiltrated with a yellowish gelatinous material.

(2) *Swelling of the Hock Joints and Feet.*—Occasionally the hock joints were found swollen and the swelling extended to the feet. In two cases the wing joint was involved. The swelling consisted of the same gelatinous material as noted above. The skin over the swollen joints was usually spotted and assumed a scarlet hue.

(3) *Vesicles on the Comb.*—This was the most peculiar change noted. The size of the vesicles varied from pin-head to a bean, and some were noted to coalesce. The vesicles consisted of a very thin membrane filled with a straw-coloured fluid, and the minute vesicles appeared as slight yellowish elevations on the comb.

When the membrane was removed the tissue underneath was found to be ulcerated. The appearance of the vesicles has been usually associated with a slower but invariably fatal course of infection. The following case is quite typical.

Bird No. F12, inoculated on 22/3/34, with a Seitz filtrate of liver and spleen emulsion in saline taken from the body of a natural case which had been buried for four days. On 28/3/34 the inoculated bird was noted to be depressed. On 29/3/34 vesicles were noted on the comb, most of them attaining the size of a bean and having some tendency to coalesce. The bird died on 30/3/34 and when examined showed typical P.T.V. *post-mortem* changes.

Swelling of the head parts when these are present are considered by Beaudette,[7] Stubbs,[8] and other American workers as features of diagnostic importance in fowl-plague. Among European workers, Gerlach and Michalka[9] have noted the same changes. Beaudette[10] has also noted the appearance of the vesicles on the comb and associates them with mild, not necessarily fatal, fowl-plague infection.

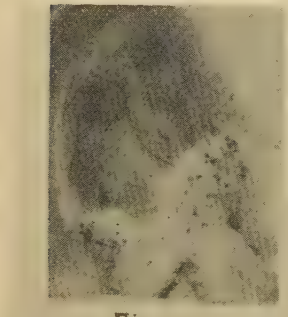

Fig 1

Fig 2

FIG. 1. Hæmorrhages at the fat around the heart.
FIG. 2. Hæmorrhages in the proventriculus.

(III) *Post-mortem Changes.*

The *post-mortem* of bird No. N5, a case of spontaneous infection which was brought alive to the laboratory and succumbed 24 hours later, may serve as an example.

Rigor mortis complete; condition good; purplish discoloration of the comb. Wattles and ear lobes swollen. Cloaca slightly prolapsed and few pin-point hæmorrhages on its m.m. Minute hæmorrhages in the conjunctiva. No appreciable exudate in the buccal cavity; few minute hæmorrhages under the tongue. Gelatinous infiltration of the s.c. tissues and few hæmorrhages in the breast muscles. Petechiæ on the serous membrane inside the base of the breast bone. Pericardium distended with a slight amount of serous fluid. Pin-point hæmorrhages in the fat around the heart, extending to the large vessels (Fig. 1). Lungs slightly œdematous and hæmorrhages on the parietal surface. Liver congested; spleen slightly swollen, pale red in colour. Kidneys

very much swollen, hæmorrhages in the renal fat. Few hæmorrhages on the abdominal wall. Hæmorrhages in the external fat of the proventriculus and gizzard. Few pin-point hæmorrhages in the larynx. No appreciable exudate in the trachea. Pin-point hæmorrhages in the m.m. of the œsophagus at the entrance to the proventriculus. Hæmorrhages on the m.m. of the proventriculus and ecchymosis at the junction with the gizzard (Fig. 2). The cuticle of the gizzard easily removed, few hæmorrhages under the cuticle. Diffuse hæmorrhages in the duodenum.

Bird No. N5 showed most of the characteristic P.T.V. *post-mortem* lesions. This was not always the case with other birds which died from the natural or, especially, from the experimental infection. Thus from the first 73 birds examined 45·2 per cent. showed swelling of the head parts, 46 per cent. gelatinous infiltration of the s.c. tissues, 19·8 per cent. hæmorrhages in the buccal m.m., 45·2 per cent. hæmorrhages in the conjunctiva, 60·2 per cent. hæmorrhages at the base of the breast bone, 87·5 per cent. hæmorrhages in the fat around the base of the heart, 56 per cent. hæmorrhages on the external surface of the proventriculus and gizzard, 80 per cent. hæmorrhages on the m.m. of the proventriculus, 45·3 per cent. hæmorrhages under the cuticle of the gizzard, 45·3 per cent. hæmorrhages on the abdominal walls, 61 per cent. hæmorrhages on the m.m. of the duodenum, 34 per cent. œdema of the lungs, 19 per cent. had a greyish layer over the surface of the lungs, 20·8 per cent. fluid in the pericardium, 26 per cent. hæmorrhages in the larynx and trachea, and about 6 per cent. had no lesions.

The hæmorrhages in the proventriculus (Fig. 2) and elsewhere differed in their intensity. They could be numerous or only very few. They could be easily observed at the junction of the proventriculus and gizzard, in which location they were as a rule more diffuse. In some cases only after the slime deposit covering the m.m. of the proventriculus had been washed off could a single or a few minute hæmorrhages be revealed.

From the above description of *post-mortem* lesions it can be noted that the presence of hæmorrhages is the constant characteristic lesion produced by the P.T.V. The hæmorrhages vary in intensity and distribution, but correspond in a general way to those peculiar to fowl plague. According to Rice,[11] linear and punctiform hæmorrhages are revealed on autopsy in characteristic cases of the disease, while many authors attribute diagnostic importance to hæmorrhages in the proventriculus when such are present.

(IV) *Mortality.*

Under field conditions a 100 per cent. mortality (native birds mainly) has been recorded by the author. In the course of this work 160 birds (S.C.W.L.) were inoculated with known virulent material and none survived.

According to Todd,[12] in countries which have been free from outbreaks of fowl plague for some time the domestic bird is very susceptible, and artificially inoculated birds also invariably succumb.

(V) *Ætiology.*

As a rule, cultures made from the heart, liver and spleen on agar, blood-agar and broth, respectively, remained negative. From one naturally infected bird a culture of *P. avicida* was isolated. At first it was thought that this organism was associated with the disease, but when inoculated into normal birds it failed to set up infection. Moreover, filtration experiments showed definitely that the ætiological agent of the epidemic was a filterable virus. The following experiment illustrates the point.

On 29/4/34 6 grammes of liver, removed from the bird No. B1 immediately after death, were ground in 30 c.c. of saline. The resulting emulsion was centrifuged slightly, and 10 c.c. of the supernatant fluid added to a 24 hours broth stock culture of *P. avicida* and transferred to a Seitz filter. Filtration was carried out under negative pressure supplied by a water pump, at room temperature. Filtration commenced in about one minute, and 5·5 c.c. filtered in 20 minutes. Sterile broth was inoculated with a few drops of the filtrate; 0·5 c.c. were inoculated i.m. into a pigeon, and the rest into bird No. 406.

The broth culture remained sterile. The pigeon was unaffected, but when subsequently inoculated with the same strain of stock culture of *P. avicida* it died in 24 hours. Bird No. 406 was found dead on 2/5/34, *i.e.*, three days after the inoculation, with lesions typical of the natural disease.

In some filtration experiments the Seitz filtrate failed to set up infection. In birds infected with Seitz filtrates of liver and spleen emulsions in saline the shortest period of incubation was three days and the longest seven days.

(VI) *Presence of the Virus in Various Organs and Body Fluids.*

From the organs that have been removed from the bird immediately after death and washed in saline, a 1 : 10 emulsion in saline was prepared and tested for the presence of virus. Usually 1 c.c. of the resulting emulsion was inoculated, except in the case of adrenals, thyroid tissue and spinal cord, the dose in those cases being much smaller.

The results of these experiments are summarised in Table I. It can be noted from these data that the virus was present in all organs and body fluids of birds affected with the disease. Out of four inoculations with fæces the disease was transmitted but once. The results obtained with the P.T.V. correspond with those obtained by Todd[12] with fowl plague. Centanni,[13] working with fowl plague virus succeeded in infecting with fæcal material

four birds out of 19, while Marchaux (cited by Hutyra and Marek) thinks that fæces are free of virus.

TABLE I.

Presence of Virus in Organs and Body Fluids.

No. of Bird.	*Date of Inoculation.*	*Material.*	*Days survived.*
N5G2	25/3/34	Liver	3
B.T.	25/3/34	Kidneys	2½
R.W.	27/3/34	Spleen	2½
N3G3	28/3/34	Muscle	2
G.W.	29/3/34	Brain	2½
G.L.	30/3/34	Spinal cord	3
N3G4	31/3/34	Thyroid	2
H.A.	8/4/34	Adrenals	4
N.S.	9/4/34	Lungs	3
G.W.	27/3/34	Whole Blood	2
G.T.	27/3/34	Blood plasma	2½
W.B.T.	27/3/34	Blood cells	2
492	8/5/34	Mouth washing	3
423	20/6/34	Faeces	2½

(VII) *Infectivity of Blood.*

Several titration experiments have been carried out to determine whether or not the infectivity of the blood of birds inoculated with the P.T.V. would surpass the m.l.d. given by Purchase[6] for atypical fowl plague (Lagrange). The following protocol is an example.

(*a*) Whole blood was drawn, with a sterile syringe containing a few drops of heparin, from the heart of bird No. 48 *in extremis* and dilutions prepared in normal saline with sterile pipettes. One c.c. of each dilution was inoculated into four-month-old S.C.W.L. cockerels as follows:—

Bird No. G.B.L. received 1 c.c. of a 1 : 10,000 dilution and died on the third day. Bird No. 3G.N. received 1 c.c. of a 1 : 100,000 dilution and died on the second day. Bird No. 2G.N. received 1 c.c. of a 1 : 500,000 dilution, and was found dead on the third day.

It can be noted from the above protocol that the infectivity of whole blood from cases of the P.T.V. disease surpasses the m.l.d. of atypical fowl plague which was found by Purchase[6] to be 0·001 c.c.

(*b*) *Infectivity of Plasma and Blood Cells.*

The blood was drawn in the manner described above and centrifuged for 20 minutes at 2,500 r.p.m. The cell-free plasma was pipetted off and the cells washed three times in saline, re-suspended in volume of saline equal to the original volume of plasma and titrated as above. The results are shown below :—

TABLE II.

Dilution.	*Material.*	*Days survived.*
1 : 10	Plasma	2
1 : 100	„	2½
1 : 1,000	„	2
1 : 10,000	„	5
1 : 100,000	„	5
1 : 100,000	Cells	2½
1 : 1,000,000	„	3
1 : 10,000,000... ...	„	3

In other titration experiments the plasma was found to be infective in 1 : 10,000, but not in 1 : 100,000 dilution, and the cells were not infective in 1 : 10,000,000 dilution. It therefore appears that the limit of infectivity for plasma lies between 1 : 10,000 and 1 : 100,000, and that for cells between 1 : 1,000,000 and 1 : 10,000,000. These figures correspond with those given by Todd[12] for blood from cases of fowl plague.

(VIII) *Pathogenicity for Other Birds.*

Pigeons.

Eighteen young and twelve old pigeons were each inoculated on different occasions with 0·5 c.c. of virulent blood, or with 0·5 c.c. of 1 : 10 liver emulsion. The control cockerels that received the same material invariably succumbed within three days. All the pigeons except one remained healthy and were discharged after a period of observation of one month. One pigeon was found dead on the morning following the inoculation, but its death could not be attributed to the effect of the virus.

Ducks.

Eight young Khaki-Campbell ducks, two-and-a-half months old, were each inoculated with 2 c.c. of virulent blood, and kept in the same enclosure with eight uninoculated ducks. A pullet that received the same material succumbed on the third day. All the ducks remained healthy and were discharged after a period of observation of 40 days.

Geese.

One young goose received 2 c.c. of virulent blood i.m. together with a control cockerel which received the same material. The inoculated goose was kept in the same enclosure as the inoculated cockerel together with another young uninoculated goose. The cockerel died on the second day. The inoculated goose was noted to be sick on the fifth day, showing extreme nervous symptoms. It was found dead on the sixth day. The only changes noted on *post-mortem* were reddening of the conjunctiva and a gelatinous infiltration of the s.c. tissue around the head. The uninoculated goose was kept under observation for two-and-a-half months without showing any symptoms.

Sparrow.

Two young *P. domesticus* were each inoculated with 0·2 c.c of virulent blood i.m. and kept in the cage together with an uninoculated control. One bird was found dead on the fifth day. The other inoculated bird showed extreme nervous symptoms, not unlike those exhibited by pigeons fed on a vitamin B deficient ration. This bird was sacrificed, and a 1 : 10 emulsion from liver and brain in normal saline prepared. One c.c. of this emulsion was inoculated into a cockerel. It died from the typical disease on the third day following the inoculation.

Investigators differ with regard to the susceptibility of pigeons to infection with fowl plague virus. The work of Doyle[14] and Purchase[6] clearly indicates that young and old pigeons as well as ducks are not susceptible. Natural outbreaks of the disease have been observed among geese.[15] Several investigators have found[16-17] that while young geese are susceptible, showing extreme nervous symptoms when inoculated i.m., old geese cannot be infected by this route, but succumb to intracerebral inoculation. According to Todd[12] it is possible to infect the sparrow among other wild birds.

The pathogenicity of P.T.V. does not, therefore, differ from fowl plague virus, for we have failed to infect pigeons and ducks, but have found young geese and the sparrow susceptible.

(IX) *Routes of Infection.*

(*a*) The disease could be easily reproduced by s.c., i.m., i.v. inoculations, and by inoculation into the feather quill. It was also reproduced with ease by dropping a few drops of virulent blood on the cloaca, conjunctiva, external nares, nasal cleft, and trachea.

(*b*) *Transmission by Ingestion of Virus.*

Two experiments were carried out with a saline emulsion of virulent liver material introduced directly into the crop by means of a sterile pipette. The results were entirely negative. Two weeks following the introduction of the virus the birds were tested

for immunity and succumbed. In subsequent experiments gelatin capsules containing known virulent liver material were used. Thus bird No. 110 received one gelatin capsule containing about 1 gramme of liver, bird No. I.T. received two gelatin capsules containing double the amount of virus, and bird No. 404 received three gelatin capsules. All the birds remained healthy, and when tested for immunity 25 days later they succumbed to the infection.

The conditions of the experiment were then varied, and instead of one single feed, the gelatin capsules were fed over a longer period. Thus bird No. 482 received between 27/4/34 and 8/5/34 eight gelatin capsules, each containing about 1 gramme of virulent liver material. The bird remained unaffected, and when tested for immunity succumbed to infection.

However, when the infected liver material was fed in the grain the bird contracted the infection. Thus bird No. 475 received on 5/5/34-6/5/34 and 8/5/34 pieces of liver material in the grain. The bird succumbed on the morning of 12/5/34 with typical P.T.V. *post-mortem* lesions.

Todd[12] reports that the results of feeding with fowl plague virus are irregular, and Doerr and Zdansky[18] succeeded in transmitting the disease by feeding in six out of 40 tests. Our experiments indicate that the virus does not penetrate through the intestinal mucous membrane but the infection may occur through the naso-laryngo mucous membrane, thus explaining the irregular results obtained after feeding birds with virus.

(*c*) *Transmission by Contact.*

In the first set of four experiments one healthy bird was kept in contact with one inoculated bird in the same cage. Upon the death of the inoculated bird it was left in the cage for about six hours, and removed afterwards. The uninoculated bird was left in the cage for a period varying between ten and 23 days. In no case did the healthy bird contract infection, and when tested for immunity it succumbed to the infection.

In a second set of two experiments the dead bird was replaced on the same day by a newly-inoculated bird. The following is an example: On 25/3/34, a healthy S.C.W.L. cockerel, No. 49, was placed together with an inoculated bird, No. 3G2, in the same cage. 3G2 was found dead in the cage on the morning of 28/3/34 and was replaced by 1G5, which died in turn on the morning of 30/3/34. From this date No. 49 was kept in the cage up to 8/4/34, when it was tested for immunity, and died on 12/4/34 as a result of the inoculation.

In a third set of two experiments the number of infected birds placed in contact with healthy birds was always more than one. The following is an example: On 15/6/34 one healthy cockerel, No. 52, was placed in contact with three infected birds. The infected birds died during the night of 17/6/34 and were replaced

on 18/6/34 by two other infected birds. These were found dead on 20/6/34. No more infected fowls were added, and bird No. 52 was found dead in the cage on 24/6/34. A test inoculation of its liver killed the bird on the third day with typical P.T.V. lesions.

It can be noted from the above that we have failed to transmit the disease when a healthy fowl was kept in contact with one infected bird; nor could we do so by keeping it in contact with two infected birds at different times. However, when the healthy bird was kept in contact with more than one infected bird and the period of contact was prolonged by the addition of newly-infected birds, contact infection took place.

American workers[7-8] first pointed out that contact infection does not take place in fowl plague. This observation was confirmed by Todd,[12] who said that it occurred rarely under laboratory conditions. Doyle[14] was also able to confirm the above observation, but pointed out that contact infection takes place if the intensity of the source of infection is increased. In this respect the P.T.V. virus does not differ from fowl plague virus. Experiments are now in progress on the rôle of insect vectors in the transmission of the disease and the results will be reported later.

(X) *Immunisation.*

As none of the inoculated birds survived infection, various methods have been devised for the purpose of immunisation. This work is still in progress and the results are to be reported later.

Todd's Vaccine.

One of the first methods tested was the phenol-glycerine vaccine recommended by Todd[19] for fowl plague. Nine birds have been vaccinated; none of them showed the slightest disturbance as a result of three vaccine doses, but when tested for immunity by i.m. inoculation of virus all except one bird, No. 480, succumbed. This bird when tested for immunity went through a severe attack of the disease; it refused food, was depressed and weak for about one week, and then it slowly showed considerable improvement. When tested again for immunity it proved resistant. When given another inoculation on a subsequent occasion it remained resistant.

Potency of the Serum.

A week after the last inoculation 10 c.c. of blood was drawn from the heart of bird No. 480 by means of a sterile syringe and the serum collected. Cock No. P.I. received 2·5 c.c. of this serum in one leg and 1 c.c. of virus in the other. A control cockerel that received the virus alone died on the second day, while bird No. P.I. remained unaffected. Five days following the simultaneous inoculation, bird No. P.I. together with a control bird received another inoculation of virus. The control died on the third day, while bird No. P.I. remained in excellent condition. Subsequently birds No. 480 and P.I. each received three inocu-

lations of virus at weekly intervals, and at the end of this period 14 c.c. of blood was drawn from the heart of bird No. 480 and the collected serum used in the following experiments.

(XI) *Neutralisation and Cross-Immunity Tests.*

Owing to the fact that fowl plague is not endemic in Palestine, the importation of fowl plague virus even for experimental purposes is strictly prohibited. It was therefore necessary to carry out the experiments reported below in the Veterinary Pathological Laboratory of the Egyptian Government, and in the Veterinary Serum Institute, Cairo, Egypt.

Fowl Plague Virus, Egyptian Strain.

Three strains of fowl plague virus were isolated and maintained by the Veterinary Pathological Laboratory, Egyptian Government. Although these strains differ in respect to the m.l.d. and the period of survival of birds after inoculation, they were found by Carpano[20] to be identical with the classical virus of fowl plague. In the experiments reported below we have used a fowl plague virus isolated on 29/11/32, supplied to us by Dr. Awad of the Government Pathological Laboratory, Cairo. This strain corresponds to strain C.,[20] for it invariably killed inoculated chickens within 36 hours.

Petach-Tikva Virus.

Virulent blood in sealed ampules was taken to Egypt, and passaged through native fowls before use.

Immune Serum.

(*a*) Serum of bird No. 480 which had been immunised against the P.T.V. by Todd's phenol glycerine method.

(*b*) Immune bird No. P.I., immunised against the P.T.V. by simultaneous inoculation of virus and serum (480).

Normal Birds.

Native Egyptian fowls were used, and were being kept in isolation for ten to 14 days before inoculation.

Technique.

The virus used was in the form of blood which was subjected to freezing and thawing in a salt and ice mixture, and diluted 1 : 10 in normal saline. 0·5 c.c. to 1 c.c. of the virus was placed in sterile test tubes and an equal amount of Petach-Tikva immune serum added. The control tube contained the same amount of virus, to which an equal amount of normal serum was added. The tubes were incubated for about three hours at 37° C., left in the dark at room temperature for three hours, and the contents used for inoculation. The bird supplying the normal serum was kept under observation for six days after the blood was drawn. It was then inoculated with fowl plague virus and succumbed.

The results of the experiments are summarised below.

Experiment 1, 10/9/34.

Tube 1. 0·5 c.c. Petach-Tikva virus, plus 0·5 c.c. Petach-Tikva immune serum (480).

Tube 2. 0·5 c.c. Petach-Tikva virus, plus 0·5 c.c. normal serum.

Results.

Bird No. 1,846, which had been inoculated with the contents of tube No. 2, was found dead on the morning of 13/9/34. Bird No. 1,884, which received the contents of tube No. 1, remained healthy.

Experiment 2, 12/9/34.

Tube 1. .1 c.c. of Egyptian fowl plague virus, plus 1 c.c. Petach-Tikva immune serum (480).

Tube 2. 1 c.c. of Egyptian fowl plague virus, plus 1 c.c. normal serum.

Tube 3. 1 c.c. of Egyptian fowl plague virus (unincubated).

Results.

Bird No. 1,873, which received the contents of tube 2, was found dead on the morning of 14/9/34. Bird No. 1,873, which received the contents of tube No. 1, remained unaffected. Bird No. P1, which was immunised against the Petach-Tikva disease, received the contents of tube 3, and remained healthy.

Experiment 3, 14/9/34.

Tube 1. 1 c.c. Egyptian fowl plague virus, plus 1 c.c. Petach-Tikva immune serum (480).

Tube 2. 1 c.c. Egyptian fowl plague virus, plus 1 c.c. normal serum.

Results.

Bird No. 1,875, which received the contents of tube No. 2, was found dead on the morning of 16/9/34. Bird No. 1,874, which received the contents of tube No. 1, did not develop the disease.

The results of Experiment I indicate that the serum from bird No. 480 immunised against the P.T.V. and neutralised the P.T.V. The results of Experiments II and III indicate that the same serum (480) neutralises known fowl plague virus. Experiment II further indicates that a bird immune to the disease produced by the P.T.V. is also immune to fowl plague virus.

Summary and Conclusions.

The author places on record experimental data showing that the virus isolated from cases of "*Acute Cell-Inclusion Disease*" during an outbreak in Petach-Tikva, Palestine, produces in fowls a disease which is similar in every respect to that produced by known fowl plague virus. Furthermore, serum obtained from birds immunsed against the Petach-Tikva virus neutralises known fowl plague virus, and birds immune to the Petach-Tikva disease are found to be immune to fowl plague. These results indicate that the two viruses are identical.

Acknowledgment.

We take this opportunity to express our thanks to the Acting Director of Veterinary Service, Cairo ; the Director of the Veterinary Pathology Laboratory, Cairo ; The Director of the Veterinary Serum Institute of the Egyptian Government, Cairo ; and to the members of the staff, for their kind co-operation in enabling me to carry out the cross immunity tests in their laboratories.

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A STUDY ON "*CELL-INCLUSION DISEASE*" IN FOWLS

II. On the Diagnostic Value of the "Chromatic-Inclusions" in the Leucocytes

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In the previous paper[1] the author has placed on record experimental proofs that a virus isolated from cases of "*acute cell-inclusion disease*" produced in fowls a disease which is similar in every respect to that caused by known *fowl-plague* virus. The author also demonstrated that birds immune to the virus obtained from cases of "*acute cell-inclusion disease*" remain healthy after inoculation with known *fowl-plague virus*, and that immune serum from these birds neutralises known *fowl-plague* virus.

"*Cell-Inclusions*" have also been reported in birds suffering from mild as well as from chronic, relatively benign, infections.[2] These "inclusions" have been found particularly in cases which showed symptoms identical with those noted in spirochætosis but failed to respond to treatment with known specific drugs, and in which no demonstrable causative agent could be detected.

The presence of "leucocytic-inclusions" in these cases led the author to believe that these "inclusions" possessed no pathognomic importance, but were simply a by-product of various types of infections.

In this paper field and laboratory observations are reported concerning "*chromatic inclusions in the leucocytes.*" These observations have extended over a period of three years, including the examinations of numerous blood smears received for diagnostic purposes, and investigations of specific outbreaks.

Field and Laboratory Observations.

(1) *The Presence of "Leucocytic-Inclusions" in Infected Birds.*

Macfie[3] was first to describe leucocytic-inclusions as occurring in "big cells with kidney-shaped nucleus and blue staining cytoplasm"; the inclusions varied from minute granules lying in vacuoles to large chromatic spheres; annular and club-shaped bodies were also observed. Subsequently Adler[4] described similar inclusions in all types of leucocytes except eosinophiles and mast cells. Gilbert *et al.*[2] noted that "*inclusions*" occur in the polymorphonuclear leucocytes, and only in two cases were the "*inclusions*" observed in mononuclear cells.

Macfie[3] and Adler[4] considered the "*inclusions*" as the specific causative agent of the disease. Knowles, Acton, and Das Gupta,[6] however, after examining Macfie's plates came to the conclusion that the "*inclusions*" are products of karyorrhexis and chromatolysis.

Inclusions identical with those described by Adler[4] have been noted by the author in the course of his studies of such well-known specific infections as spirochætosis, leukemia, occasionally in the late stages of fowl-plague, and rarely in severe Egyptionellosis. Similar inclusions were noted by Stubbs[6] in America in blood smears taken from birds affected with leukemia. The inclusions noted by the author are shown in the accompanying photographs (p. 299).

It is open to surmise, however, that the inclusions might have been present in birds suffering from mixed infection. Adler[4] noted that "*Cell-Inclusion Disease*" may be complicated by leukemia, and Gilbert *et al.*[2] noted that the disease may be complicated by such known infections as spirochætosis, fowl typhoid, and fowl cholera. Chromatic inclusions identical with those described by Adler[4] were most often noted by the author to occur in conjunction with spirochætosis. Early in our work such cases have been diagnosed as mixed infections. However, observations in the field were not consistent with such a diagnosis. When an affected flock in which mixed infection had been diagnosed was treated with neosalvarsan the sick birds recovered completely. Moreover, the apparently healthy birds of the same flock which were also injected with neosalvarsan remained well for some time but later developed spirochætosis with similar inclusions and responded to early treatment with spirocid.

Occasionally blood smears received from an affected bird showed "*inclusions*" alone but no spirochætes. As a rule a laboratory diagnosis of "*cell-inclusion*" *disease* was made in these cases. When, however, in such cases the flock was visited and blood smears taken from other affected birds, spirochætes were always found. As the owners usually send blood smears for examination from the most badly affected birds, often prior to death, one fails to detect spirochætes, because the blood smear was taken after "lysis." Similar findings were also noted when blood smears sent for examination were taken early in the "after-phase" period, which according to Knowles *et al.*[7] may extend from one to 52 days, or after the bird was given a globule of spirocid. In such cases "inclusions" alone but no spirochætes were found.

Ten such outbreaks have been investigated and the results of these investigations are summarised in Table I. On several occasions the owners have sent material to other laboratories. We have included in Table I the official diagnosis of the other laboratories for the sake of comparison.

It can be noted from Table I that in ten flocks of 2,565 birds in which a diagnosis of mixed or pure infection was rendered, contrary to what might have been expected, the birds responded to treatment with neosalvarsan or spirocid, and complete recovery of the sick took place within 36 hours. In two flocks treated with neosalvarsan and one with spirocid, a total of nine birds succumbed

TABLE I.

Outbreak.	*No. of birds.*	*Per cent. sick.*	*Microscopic finding.*	*Diagnosis.*	*Other diagnosis.*	*Treatment.*	*Death within 24 hours.*	*Result.*
Kt.	250	20-25	Spirochaetes and inclusions	Mixed infection	Mixed infection	0·02 grs. neosalvarsan I.M.	4	Recovered
En.	600	30-40	,,	,,	Cell inclusion	,,	–	,,
G.	450	20-30	,,	,,	—	,,	–	,,
E.H.	200	15	,,	,,	—	,,	2	,,
K.Y.	80	10-15	,,	,,	—	,,	–	,,
R.	400	10-15	,,	Spirochaetosis	Cell inclusion	0·25 grs. spirocid per os.	3	,,
M.J.P.	200	20	,,	,,	—	0·02 grs. neosalvarsan I.M.	–	,,
MP.P.	200	10-15	,,	,,	Cell inclusion	,,	–	,,
N_1	150	20	,,	,,	,,	0·25 grs. spirocid per os	–	,,
NL.	35	10	,,	,,	—	0·02 grs. neosalvarsan I.M.	–	—
Total	2,565						9	

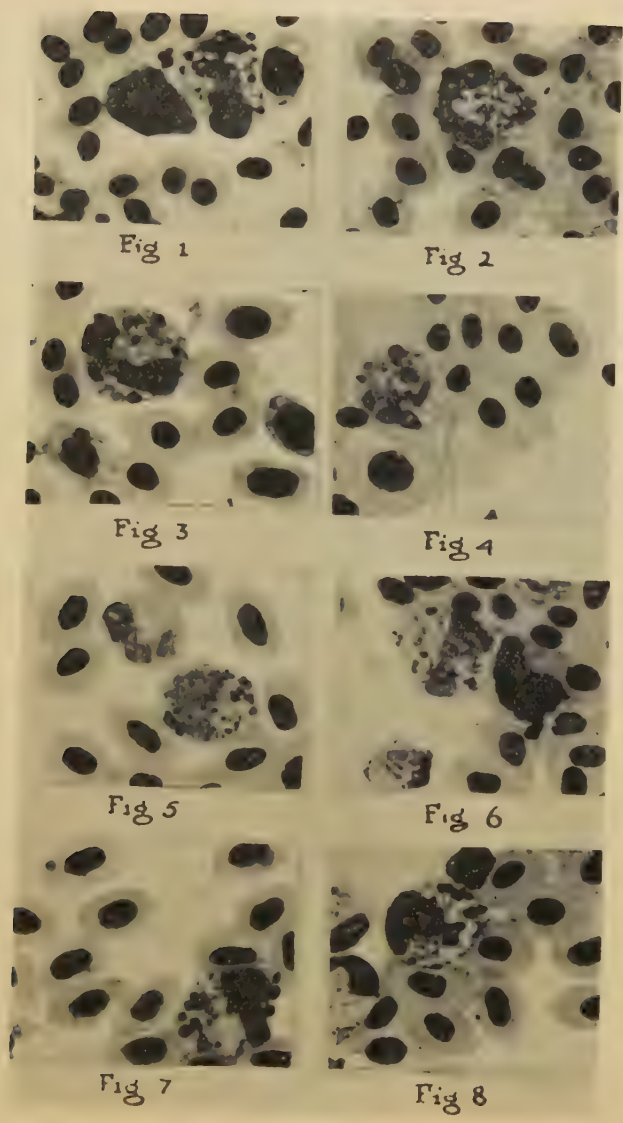

FIG. 1-5.
Leucocytic inclusion noted in blood smears taken from birds affected with spirocætosis. Figs. 4 and 5 : spirochætes can be seen.

FIG. 6.
Leucocytic inclusions noted in blood smears taken from a bird inoculated intravenously with large dose of liver and spleen emulsion.

FIG. 7.
Leucocytic inclusions noted in blood smear taken from a bird which was experimentally inoculated with *Egytuonella pullorum*. One erythrocyte contains *E. pullorum*.

FIG. 8.
Leucocytic inclusions noted in blood smear taken from a bird with Fowl-Plague, prior to death.

All preparations are stained with Giemsa sol. and magnification × ca. 900.

after the treatment. These birds were *in extremis* when treated and it was not expected that they would be benefited by the treatment. Upon *post-mortem* examination, these birds showed typical lesions of spirochætosis. No fresh cases were known to occur in the neosalvarsan treated flocks for some time, and the owners treated new cases, as soon as they appeared, with spirocid with good results. In outbreak MP.P. recovery of the sick birds took place within 36 hours, and according to the owner fatal cases began to occur again in six weeks' time after the treatment with neosalvarsan, but owing to the author's absence from the country the cause of mortality could not be investigated personally.

"*Bayer Spirocid Globules*" are most commonly used in Palestine to combat spirochætosis. Freund[8] in an extensive study on the effect of spirocid showed that the drug acts specifically against the infection when given in doses of one to two globules. It was, however, repeatedly observed by us that, whereas the drug is 100 per cent. effective in curing early cases of spirochætosis, it often fails to do so when administered in the late stages of the disease. Birds nearing lysis, or immediately after lysis, when treated with spirocid usually die shortly after the administration of the drug. Birds in the after-phase period fail to respond to a single treatment, but usually recover after a more prolonged treatment with the drug. Consequently one may say that the fact that birds showing definite symptoms of spirochætosis fail to respond to treatment with specific drugs does not in itself exclude spirochætosis infection.

(2) *Tranmission Experiments.*

On various occasions attempts were made to transmit what appeared to be "*chronic cell inclusion disease.*" Blood, liver and spleen from birds which showed inclusions alone, or from what appeared to be a mixed infection, were used. The results were either entirely negative or spirochætosis with characteristic "*inclusions*" developed. In the latter case, after treatment with neosalvarsan recovery took place, and after one to two days the "inclusions" disappeared spontaneously, and were not observed again in blood smears taken from these birds daily for a period of about 30 days. Moreover, birds inoculated with filtrates of the blood or organ emulsions, filtered through a Seitz filter, gave consistently negative results.

Fowls inoculated intravenously with large doses of normal liver and spleen emulsions in saline showed a rise in temperature within 24 hours after the inoculation and had "*inclusions*" in the leucocytes. This reaction was transitory and may be rather attributed to the influence of the inoculated foreign protein material than to any specific action of a virus. The same material heated to 70° C. for half-an-hour and inoculated in the same manner produced the same results. Furthermore, when the fever subsided

the bird appeared quite healthy, and again showed " leucocytic inclusions " when inoculated with similar material.

(3) *Cross-Immunity Tests.*

In a recent epizoötic we selected, from among Leghorn fowls from which numerous blood smears have been examined, several badly affected birds and divided them into the following groups :—

(*a*) Four birds which showed " inclusions " only.

(*b*) Two birds which showed " inclusions " and a small number of spirochætes.

(*c*) One bird which showed " inclusions " and agglomeration of spirochætes.

After treatment with spirocid one bird of each of groups (*a*) and (*c*) died within 24 hours and the others recovered. The latter were kept away from ticks or any other infection for 30 days, when they, together with a healthy control bird, were inoculated i.m., with spirochætal blood. All birds except the control resisted infection. They were then inoculated with the Petach-Tikva Virus[1] obtained from a case of " Acute Cell-Inclusion Disease." All the birds died within 48 hours, showing the typical Petach-Tikva Virus *post-mortem* lesions.

Summary and Conclusions.

The observations reported above indicate that chromatic inclusions identical with those described by Adler[4] are found in a variety of infections. These leucocytic cell-inclusions were regularly encountered in birds shown to be infected with spirochætosis. Attempts to demonstrate a specific virus in birds suffering from a so-called " chronic cell-inclusion disease " gave negative results.

Although the observations placed on record in this paper do not exclude the possibility of the existence among poultry in Palestine of a specific virus disease known as " Cell-Inclusion Disease," it seems clear that no pathognomonic value can be attached to the presence of such " leucocytic-inclusions."

Acknowledgment.

We take this opportunity to express our thanks to Prof. Adler for his kindness in examining some of the material.

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THE STUDY OF AVIAN BIOLOGY IN RELATION TO SCIENTIFIC PROGRESS

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The biology of the domesticated fowl is usually studied for its practical applications; nevertheless, it deserves attention on the part of qualified investigators, not only as a stepping stone to research in comparative pathology, but also for its intrinsic interest.

Even a rapid retrospect of the History of Biology furnishes several examples which reveal that experiments and observations made on avian material have been the starting point of fruitful research, leading to remarkable advances in general and applied knowledge in the realm of biological sciences.

Embryology.

Already in the writings associated with the name of Hippocrates (460-370 B.C.) we find allusions to the development of the chick in the egg, from the second day to the time of hatching, *e.g.*, it is said: "He who has not made these observations before, will be amazed to find an umbilicus in a hen's egg." Then Aristotle (384-322 B.C.) described in περὶ ζώων μορίων = "about the parts of animals" the shape of the chick embryo on the third day of incubation, whilst the early appearance of the rhythmically throbbing heart is also mentioned. Although for many centuries afterwards the development of the chick in the egg continued to be a subject of considerable philosophical curiosity, progressive observations did not appear till 1573, when Volcher Coiter (1534-1576 or 1590), in a book on human anatomy, introduced a chapter on generation in the egg, with woodcuts which may be considered the first accurate embryological delineations. Volcher Coiter's teacher, Ulisse Aldrovandi of Bologna (1522-1605), in his "*Ornithologia*" printed in Bonn in 1597, attempted to describe all known birds, among which he included bats and harpies, and illustrated a chick in the act of hatching out. These somewhat incomplete figures were followed by the drawings of Fabrizio da Acquapendente (1537-1619) in "*De formatione ovi et pulli*," which appeared posthumously in 1621, and though otherwise a great advance on previous delineations yet contained two erroneous illustrations (*b* and *c*, third and fourth days of incubation), for which the author possibly was not responsible. Artistically superior to these are the drawings of Marcello Malpighi (1628-1694) in his Royal Society memoirs, entitled "*De formatione pulli in ovo*" (1673) and "*De ovo incubato observationes*" (1689).

These exact delineations avoided illustrating some misleading observations which Malpighi made on an egg which must have been incubated and which led him to believe that he could discern the form of an embryo in an unincubated egg; thus suggesting the doctrine of " preformation " or " emboîtement " which persisted well over a century and supposed that every embryo contained encysted the formed features of succeeding generations. This view influenced philosophical thought to a considerable extent, till it was disproved by Caspar Friedrich Wolff (1733-1794), who revived Harvey's doctrine of epigenesis or building up of parts.

Still, these exact delineations certainly helped to place the iconography of embryology on a firm scientific basis and furthered the study of a subject about which, in a chapter entitled " The efficient cause of the chicken is hard to find out," William Harvey (1576-1657) had despondently remarked: " Neither the school of physitians, nor Aristotle's discerning brain, have disclosed the manner how the cock and its seed, doth mint and coine the chicken out of the egg."

Harvey himself made numerous observations on the development of embryos in eggs and it is noteworthy that the frontispiece of his second great work, " *De generatione animalium,*" represents Zeus holding an opened egg, from which are issuing several arthropods, also a snake, a lizard, a dolphin, a bird, a stag and a child : on the egg-shell there is an inscription, " *Ex ovo omnia.*" This is an example of intelligent anticipation on the part of Harvey, who had never seen a mammalian ovum. In any case the inscription was later happily combined with the aphorism of Francesco Redi (1626-1694), " *Omne vivum ex vivo.*"

During the eighteenth century the embryological observations of the practising court physician, Felix Vicq d'Azyr (1748-1794), deserve to be mentioned, for he described the germinal vesicle in the egg whose significance was recognised by Johannes Evangelista Purkinje (1787-1869) and illustrated by him in 1825 in " *Symbolæ ad ovi avium historiam ante incubationem.*" The anatomical proof of the aphorism " *Omne vivum ex ovo* " went through a stage of error, when the ovarian follicles described in 1672 by Regnier de Graaf (1641-1673) were considered mammalian ova, though he found some difficulty in explaining why the earliest embryos were smaller than the follicles. His views were opposed by Leeuwenhoek (1632-1723) and also by Antonio Vallisnieri (1661-1730), whilst Albrecht von Haller (1708-1777) held that in mammals the embryo arose by the fertilisation of a fluid. Later, the problem was solved as the outcome of the patient and accurate microscopical examinations of Carl Ernst von Baer (1792-1876), who detected the ova in mammalian Fallopian tubes and whose observations were published in the treatise " *De ovi mammalium et hominis genesi* " (1827).

This notwithstanding, complete agreement had to await the establishment of the cell-theory by Theodor Schwann (1810-1882), who recognised the egg as one cell and the germinal vesicle as its nucleus—a view confirmed decisively in 1861 by Carl Gegenbaur (1826-1903). Even so, Wilhelm His (1831-1904) maintained for some time after that not all cells were derived from the ovum directly, but that notably the blood and connective tissues originated from maternal leucocytes which had migrated into the yolk whilst yet in the ovary.

A further achievement of von Baer is his germ layer theory which may well rank as one of the most valid generalisations in the whole field of embryology. In this important observation he was guided by the previous work of Caspar Friedrich Wolff, in the memoir entitled "*De formatione intestinorum*" (1768), which deals with the embryonal development of the chick bowels and describes the rolling up of the leaflike embryonic layers.

It may be recalled that in 1817 Christian Pander (1793-1865), assisted by von Baer determined three embryonic layers in incubated eggs; these were then described in Burdach's Treatise on Physiology. These observations were later extended in the direction of comparative embryology in the great work of von Baer: "*Ueber die Entwickelungsgeschichte der Thiere. Beobachtung und Reflektion*" (1828).

From these beginnings, morphological comparative embryology was advanced by Albert von Koelliker (1817-1905) and reached its apogee with the master work of Francis Maitland Balfour (1851-1882).

The physical and chemical aspects of the science should not be overlooked; evidence of the remarkable extent of these is to be found in the three volumes of "*Chemical Embryology*" by Joseph Needham, which appeared in 1931. It was Sir Thomas Brown (1605-1682) who first experimented to ascertain the constituents of the eggs of hens, frogs and fishes. Investigations of a similar nature on mammalian embryos followed about a quarter of a century afterwards and are connected with the name of the physician Walter Needham (?1631-?1691) in "*Disquisitio anatomica de formatu fœtu*" (1667).

Comparative Anatomy.

In the year 1555 appeared a monograph on birds, in which Pierre Bélon (1517-1564) compared the erect posture of birds (which, however, is particularly noticeable in the Old English Game fowl) and man and then illustrated and construed nearly as possible the homology between the various bones of bird and man. Bélon, a travelled observer, may be considered to have heralded the advent of Comparative Anatomy, though previous to this treatise, Leonardo da Vinci (1452-1519) in his notes, had illustrated the hind leg of a horse and compared it bone for bone and

muscle for muscle, with the leg of a man and also tried to construct an analogy between the chick in the egg and the fœtus in the womb. (Drawings at Windsor Castle.)

Here it may be mentioned that Leonardo da Vinci, who was an accomplished horseman (refer to Vasari, "*Vite illustri pittori*"), represented the external anatomy of the horse in many accurate drawings; it is thought that these examples may have inspired a lawyer and Senator of Bologna, Carlo Ruini, to write (or perhaps purchase the manuscript?) a treatise on the anatomy, diseases and their treatment, of the horse, amply illustrated with exact and artistic designs of the internal organs. This book was printed in 1599, after the death of Ruini (date unknown). The dedicatory preface is by the printer, Gasparo Bindoni of Venice; it states that the text was obtained from the heirs of Ruini, among which the son Ottavio is mentioned. Very little is known about Carlo Ruini, except that he was the son of a lawyer and a lawyer himself; the preface does mention that he was a writer of distinction, a lover and breeder of horses. The illustrations of the book are obviously the work of an accomplished artist, guided by a skilled anatomist; it is difficult to think of anyone combining these abilities, except Leonardo da Vinci. In examining the woodcuts one seems to recognise the vigorous and accurate line of the great master and perhaps there is justification in speculating whether Leonardo did not complete his drawings of the muscles of the horse by sketches of the inner organs and that these sketches eventually reached Ruini in Bologna. The text should also be noticed, which is clear and classical Italian, very different from the style of the contemporary book on marshalry by Filippo Scacco (1591); the veterinary chapters seem to be based on previous authors, such as Ruffo di Calabria (beginning thirteenth century), but give the impression that the author had seen the diseases he describes.

Bindoni's Venetian edition of 1599 can be admitted to be the first, for on the title page it is stated to be a new work, worthy of any Prince or Knight and very necessary to philosophers, physicians, horsemen and farriers.

It seems as if the "*Anatomia del cavallo*" were a treatise whose authorship is at present doubtful, but about which it has been said that ". . . . its astonishing originality, truthfulness and fidelity are sufficient to establish him for ever as the father of equine anatomy and the most original veterinary writer of the sixteenth century." [Sir Frederick Smith (1919), p. 215.] In chapter XII, p. 107, the book contains some sentences which show that the author had grasped the significance of the principle which worked the valves of the heart; accordingly, Ruini has been hailed as a precursor of William Harvey. Apart from this, this treatise was the first complete work in relation to equine medicine and was translated into French and German. Its illus-

trations were copied and adopted by the farrier to Charles II, Andrew Snape (?1664-?), a man of remarkable culture, who wrote "*The Anatomy of an Horse*" (London, 1683). Several of the artistically admirable copper plates are "transfers" from Ruini, but the artist has made anatomical errors in the process. This anatomy illustrates in an appendix the development of the embryo chick in the egg, following rather closely Malpighi (1673); no acknowledgment was made in either instance. Ruini recognised that the horse lacked a gall-bladder, an observation quoted by Sir Thomas Browne in "*Pseudodoxia epidemica*" (1646). Some of the information obtainable in Ruini's work is found scattered in the numerous writings of Gervase Markham (?1568-1637), an extensive compilator, and the author of some of the first books of any consequence on veterinary and agricultural matters in the English language. Markham shows a practical grasp of his subject in the description of breaking in horses and the training of fighting cocks, the latter in the "*Pleasures of Princes or the Good Men's Recreation*" (1613), of which it is stated: ". . . . an admirable piece of work. Markham evidently wrote from intimate knowledge." (Sir Frederick Smith: "*The early development of veterinary literature, etc,*" London, 1919.)

Marco Aurelio Severino (1580-1656) in his "*Zootomia Democritæa id est anatomia generalis totius animantium opificii*" (1645) sought to trace analogies in the corresponding parts of various animals. Then Nehemiah Grew (1641-1712) in his "*Comparative Anatomy of the stomach and guts*" illustrated and compared the bowels of birds, fishes and mammals, particularly in some phases of their development. Edward Tyson (1650-1708), who graduated in Cambridge in 1678, wrote in 1699 a monograph entitled "*Orang-outang, sive homo sylvestris,*" which is considered to be the first work of consequence in comparative morphology; it appears to have originated the "missing link" catch-word which later was ascribed to Charles Darwin. The notes of the lectures of Felix Vicq d'Azyr (1748-1794) published in 1805 deals fully with such anatomical comparisons between birds and quadrupeds, thus preparing the way for Richard Owen (1804-1895), whose views were critically modified by Thomas Huxley (1825-1895) in his lectures on the "*Craniology of birds*" in 1867.

The development of Comparative Anatomy has been since furthered by several workers in many different fields; but though it may be reasonably inferred that Pierre Bélon was induced to compare the anatomy of birds and man, because both were bipeds; and the horse was the first among domesticated animals to be the subject of specialised anatomical study, it will be seen that avian anatomy has obtained a fair share of scientific attention and contributed to the common stock of progressive knowledge.

Physiology of Digestion.

Though the process of digestion had claimed the attention of Jean Baptiste van Helmont (1577-1644), Franciscus de le Boë (Sylvius) (1614-1672), Giovanni Alfonso Borelli (1608-1679), yet the first experimental study of the physiology of digestion was initated by that eclectic scientist, René-Antoine de Réaumur (1683-1757), who took advantage of the habit of certain birds in regurgitating indigestible objects. De Réaumur prepared small iron cages with sponges inside, which he gave to a pet kite and so isolated gastric juice and demonstrated its solvent properties in relation to different foodstuffs; these observations were published in a treatise entitled "*Sur la digestion des oiseaux*" in 1752. De Réaumur made several other experiments on sheep for the purpose of ascertaining whether digestion was fermentation or putrefaction; a question which was warmly debated, like the problems connected with so-called "spontaneous generation"; which, following the lead of Francesco Redi and the English Jesuit, John Turberville Needham (1713-1781) were also investigated by Lazzaro Spallanzani (1729-1799), who, incidentally, by means of ingenious tests on fowls, showed the importance of grit in relation to the grinding up of wheat in the gizzard and then confirmed the solvent properties of gastric juice, in 1782. He further asked his friend, Professor Scopoli of Pavia to analyse some gastric juice obtained by means of tubes and sponges from crows; though Scopoli failed in recognising the acid in the fluid, this chemical analysis may claim to be the first made on gastric juice. Spallanzani continued his experiments on numerous other animals and then on himself and come to the conclusion that digestion was neither fermentation nor putrefaction, but something by itself. It was only in 1824 that William Prout (1785-1850) was able to prove by means of careful distillation and analysis that gastric juice contained free HCl. A further active substance, pepsin, was discovered by Theodor Schwann in 1835. Artificial digestion was then performed by Purkinje and Pappenheim in 1838 and again by Schwann in 1840; these few names do not complete the list of those who made important discoveries connected with digestive functions, a field of research which is still being actively cultivated.

Cellular Respiration.

It was John Mayow (1643-1679) who recognised that "nitro-aerial gas" can penetrate through the porous shell of the hen's egg during incubation, and in 1749 René Antoine Ferchault de Réaumur (1683-1757) (who had constructed a most ingenious "artificial hen" or incubator) observed that embryos do not develop if the eggs are covered with varnish. John Redman Coxe of Philadelphia in 1794 ascertained the presence of oxygen in the air-cell of eggs and soon after, in 1796, Hehl found that the air-cell

contained atmospheric air and thus served for the respiration of the developing embryo. The composition of the air chamber in eggs is not yet fully ascertained, because remarkable differences occur, especially in relation to oxygen content according to time and numerous other functions. The name of Spallanzani (1729-1799) is connected with the posthumous publication in 1803 of the fact that eggs during incubation give off and take in gases. Nevertheless, several other experimenters, such as Erman (1818), Towne (1839), Viborg (1840), denied this assertion, which on the other hand was defended by Geoffroy St. Hilaire (1820), Everard Home (1822) and others, till the question was conclusively settled in 1834 by Theodor Schwann (1810-1882), who in his thesis entitled "*De necessitate aeris atmosphaerici ad evolutionem pulli in ovo incubato*" brought forward accurate proof of the breathing process in egg embryos. It may be said that knowledge in relation to cellular respiration gradually evolved on the basis of avian material, for Spallanzani made numerous experiments with surviving tissues such as ovaries, liver, bowels, separated from the living animal, and ascertained that tissues "breathe."

The modern view on respiration is that there is no sharp division between cellular or tissue respiration and lung breathing; so that one may briefly mention that Robert Boyle (1627-1691) performed his first experiments on a lark and a sparrow to demonstrate that life was impossible *in vacuo*. Among the first measurements of the oxidation process in nucleated cells are those made by Otto Warburg (1909) on geese erythrocytes.

Then it may be added that William Harvey (1578-1657) refers to observations on the living heart of pigeons in Chapter IV of "*De motu cordis,*" which observation may well be considered among the first made in relation to the circulation of the blood and functions of the heart; particularly in relation to the possibility of recalling "the heart from death to life," by means of "fomentation," in modern terms resuscitation by heart massage.

Cerebral and Cerebellar Physiology.

Apparently it was Franz Josef Gall (1757-1828) and his disciple Johann Gaspar Spurzheim who first suggested that some of the functions of the brain are localised in the cortex. This theory obtained some popularity in the fantastic form of Phrenology, which actually received a certain measure of qualified approval from Wolfgang von Goethe (1749-1832). The first real advance on the crude views of Gall and Spurzheim was due to Marie-Jean-Pierre Flourens (1794-1867), who made his classical observations on the experimental removal of the cerebrum or of the cerebellum in pigeons, showing the absolute maintenance of reflexes with loss of cerebration and volition in the former case and disturbance of equilibrium in the latter. On these experiments all subsequent knowledge is based.

Genetics.

The observations of Darwin (1809-1882) in connection with the inheritance of different characters and the manner of formation of races and breeds in tame pigeons, fowls, ducks, geese and peacocks were published in : " *On the variation of animals and plants under domestication* " (1882).

It is by means of breeding experiments on fowls that in 1902 William Bateson (1861-1926) ascertained that the genetic laws observed in plants by Johann Gregor Mendel (1822-1884) and rediscovered in 1900 by Hugo de Vries (1848-) were applicable to animals; observations that have been continued by R. C. Punnett and his collaborators and which have been applied to the practical needs of poultry breeding.

The advance of general biological knowledge naturally involves that the experimental suitability of a large and widespread family, such as that of birds, could not be neglected; so that the thought may arise it was only a matter of coincidence that certain important biological laws were first investigated on fowls rather than on cats or dogs, frogs or mice. In relation to Bacteriology and Experimental Medicine it is, however, noticeable that the results of researches on fowls eventually revolutionised the treatment of human and animal diseases.

Bacteriology.

The discovery of preventive inoculation by Pasteur (1822-1895) was due to the accidental observation that, during the holidays, artificial cultures of the chicken cholera bacterium, *Pasteurella avicida,* became sterile or avirulent; so that when injected, they were found to act as preventive " vaccines " against a subsequent inoculation with a virulent strain of this microbe. The attenuated antigen could be carried through many generations and still maintain its immunising property. Further work in connection with fowls was the exhibition of virulence by the anthrax microbe, when inoculated into chickens which had been artificially cooled. The interpretations of the results of both these series of observations were faulty : the immunisation of poultry against fowl-cholera has proved to be an erratic procedure, since strains of *Pasteurella avicida* seem to vary greatly in their antigenic properties. Moreover, however much one may cool fowls down, anthrax bacteria will not acquire pathogenicity in birds, whose death is due to rapid cooling, not microbes. The further development of these investigations, though well known, is too complicated to detail here, but it amounts to this : that they eventually induced the recognition of the bactericidal properties of blood serum by Nuttall in 1888; hence the triumphs achieved in relation to the prophylactic inoculation against rabies, tetanus, diphtheria,

typhoid and dysentery, may be said to have originated in the experiments made by Pasteur on fowls.

Experimental Medicine.

The parasite of bird malaria, *Plasmodium relictum*, was originally described by Grassi and Feletti (1891); its experimental transmission by means of Culex mosquitoes, accomplished by Ronald Ross in 1898, acting under the inspiration of Patrick Manson (1844-1922), furnished the link which enabled the chain of evidence to be completed in relation to the epidemiology of the disease, when correlated with the crucial experiments on man, performed at the instigation of Sambon (1865-1931). As a further development may be mentioned that beginning with Roehl (1926), the testing of new synthetic drugs for the treatment of ague is performed on canaries inoculated with bird malaria up to the present day.

If not the first, still certainly among the first protists recognised as the cause of a definite disease in man, is to be reckoned the spirochæte of relapsing fever, discovered in 1868 by Otto Obermeier (1843-1873). The detection of a similar spirochæte in geese by Sakharoff (1892) in the Caucasus, rendered possible the investigations of Marchoux and Salimbeni (1903) in Brazil, which revealed that spirochætosis in fowls was disseminated by the fowl tick, *Argas persicus*. This discovery no doubt led to the recognition by Dutton (1876-1905) and Todd (1905) of the part played by another tick, *Ornithodoros moubata*, in transmitting one variety of African relapsing fever in man. The importance to man and animals of the many tick-borne diseases which have since been described is well known.

Vitamins.

A disease in the Dutch East Indies, originally described by Jacob Bontius in 1642 as "beri-beri," was, in course of time, found to be widespread throughout the East. Its causation was considered to be in some way connected with feeding on rice; though it was remarkable that "beri-beri" or a very similar disease occurred on board sailing ships, where little or even no rice was present in the dietary, and observation in the Philippines indicated that an infantile form of the disease could develop in breast-fed infants. These are but random samples of many more similar apparently contradictory observations which were gradually explained, when Eijkman (1897 and later) found in the Dutch East Indies, that a diet of polished rice produced in fowls a condition of paralysis, due to alterations in the nerves very similar to those occurring in prisoners fed on the same decorticated grain and which had been diagnosed as "beri-beri." Later Eijkman (1906) showed that the polishings from the rice contained a substance which prevented the development of fowl-polyneuritis and in the meanwhile Gijns (1901) had obtained a similar result

by adding the bean, *Phaseolus radiatus*, to the polished rice fed to fowls. Shortly after Hulshoff-Pohl (1902) discovered that this bean acted both as a curative and preventive agent in human "beri-beri." The name "vitamine" was then proposed for the active substance by Casimir Funk (1911), who tried to isolate it from rice polishings and yeast extracts, which he then tested on pigeons and fowls. It will be seen that these researches on birds, by confirming the original findings of Hopkins in 1906 on rats and mice fed on an "incomplete" diet, paved the way for the determination of the various "accessory food factors" or vitamins, which are now recognised to be more in the nature of essential activators of foodstuffs, rather than "accessory" factors.

Oncology.

Lastly, one may mention the accurate studies of Peyton Rous (1910) in relation to the discovery and identification of a filterable "virus" as the cause of a myxo-sarcoma tumour in Plymouth Rock fowls. Very soon afterwards, Fujinami (1911) decribed a sarcoma of Japanese fowls with the same pathological properties and later a similar tumour of ducks, which exhibits, for a neoplasm, the remarkable peculiarity of being transmissible to the domesticated fowl and therefore is not strictly specific for the animal genus from which it was originally obtained. These investigations proved an incentive to numerous researches, the results of which, when correlated with experiments on other animals, we hope may yet enable us to understand more clearly than hitherto the development of cancer in man and animals.

These examples should suffice to evince that there is full justification in investigating a particular chapter of avian pathology, like that of hæmatology, in the hope of obtaining information which may lead to wider applications.

II. The Development of the Knowledge of the Functions of the Blood, or Hæmatology.

From time immemorial it has been recognised that blood is a vital fluid and numerous sayings and phrases are still in current use to show that, in the popular estimation, blood possessed many virtues, besides being singularly relevant to genetics and similar matters. Nevertheless a knowledge of the outstanding features of the blood, its circulation in the vessels and clotting out of them, together with the essential facts pertaining to respiration, was obtained in relatively modern times. Even more recent are the advances connected with the morphology of blood cells, so it can be said that hæmatology is quite a modern branch of biology, though the designation seems to have originated in 1743, when Thomas Schwenke (1694-1768) wrote a book with the ambitious title "*Hæmatologia, sive sanguinis historia.*"

During the last century the progress of hæmatology was linked with advances in relation to its medical aspects, so that morbid alterations in the composition of human blood were mostly investigated; if animal experiments were performed, it was with the purpose of reflecting the light of knowledge so obtained on the elucidation of problems connected with human pathology. The improvements in relation to differential staining methods are the outcome of contemporary researches; these have enabled critical microscopy to control the results of experimental biology, even if not to the extent of distinguishing morphologically similar but functionally different cells from one another.

Circulation and Heart Functions.

Whilst Galen (A.D. 131-200) recognised that the arteries contained blood during life, nevertheless he taught that the heart did not contract and expand actively, but was passively dilated by the rhythmical activity of the blood, whose reflux allowed the heart to collapse; moreover that pores in the ventricular septum afforded a direct communication between right and left ventricles. The heart then served the purpose of communicating heat to the blood. These views formed the galenical doctrine of the function of the blood and heart and can be said to have diverted speculation for centuries into false byways which were further obscured by ingenious theories which explained that, whilst the arteries were normally empty, except for spirit or air, they filled with blood and spurted when injured, because blood poured in to fill the vacuum. The perverted cleverness of the system which was construed from the commonplace observation that the arteries are usually empty in dead animals need not be followed in all its intricate variations, which were evolved during the Middle Ages.

A gleam of the actual facts of the circulation of the blood may be found in Vol. III, Chap. IV of "Pantagruel," [printed in 1533], where, however, it is stated that the right ventricle sends the blood to all limbs, whilst the left performs a similar function with arterial blood, whose purpose appears to be to cool the venous blood; any mention of the interventricular canals seems to be studiously avoided. That François Rabelais (about 1490-1553) should have injected anatomical discussions in a praise of debtors, is explained by the fact that after a spell of monastic life, he studied medicine at Montpellier; then removed to Lyons about 1531 and edited a direct translation of Galen and from 1534 onwards lectured there on anatomy and also at Montpellier. These dates are important, because in 1535 a Spanish medical student, by name Miguel Serveto, was in Lyons, who may well have attended the lectures of Rabelais either there or at Montpellier. In any case, Serveto, better known as Servetus (born 1509 or 1511, burnt by order of Calvin at Geneva in October, 1553), completed the manuscript of his book on "*Christianismi*

restitutio " in 1546 in which he stated quite clearly that the blood from the right ventricle proceeded to the lungs, where it commingled with the inspired air, changed its colour and was poured from the artery-like vein into the vein-like artery. The book was printed in 1553, but all known copies were destroyed, so that only two originals are still extant. The possibility of contact between Servetus and other sources of information may be considered; because it does appear remarkable that in face of opinions so universally held and taught, one man alone should have obtained such a clear conception of a complicated physiological process. We find that the anatomist Andreas Vesalius (1514-64) in his first edition of " *De fabrica humani corporis* " published in 1543 contains a passage which may indicate ironical doubt as to the existence of the galenical interventricular passages, which doubt is further reinforced in the edition of 1555.

Further we learn that Vesalius and Servetus (who at the time had adopted the name of " Villanovanus ") dissected together under the tuition of the anatomists Johann Günther (1487-1574) and Jacques Du Bois (better known as Sylvius, 1478-1555) in Paris. Even though Sylvius was a rabid galenist, it does seem as if an exchange of ideas must have taken place between Vesalius and Servetus enabling the latter to propound his views on circulation in the definite form found in his " *Restitutio* "; opinions which are totally at variance with anything taught until then, for Girolamo Fracastoro (1484-1553), who had a clear and far-seeing intellect, admitted that the function of the heart was only to be comprehended by God. A few years after, in 1559, Matteo Realdo Colombo (1516-1559) published in his " *De re anatomica* " some observations on the circulation of the blood which possibly were a clever adaptation from a manuscript of Servetus, which had been sent to Padua in 1546. Later still, in 1571, the Italian physician, botanist (and controversial theologian) Andrea Cesalpino (1524-1603), described the blood circulation in his book " *Quæstiones peripateticæ* " and confirmed it in 1593 in another book entitled " *Quæstiones medicæ.*" The remarks of Carlo Ruini (1599) have already been mentioned; it is possible that they were obtained from Cesalpino. Then there is Paolo Sarpi (1552-1623); this very active Venetian theologian left in his memoirs some cursory notes describing the circulation of the blood, but it is thought that they were inspired by Harvey, with whom Sarpi had been in correspondence.

The opinions expressed by these various authors do not detract from the merits of William Harvey (1578-1657) who, by accurate dissections of animals and many experimental observations, ascertained and correlated the functions of the heart and vascular system and demonstrated their significance; these views he then recorded in his book entitled " *Exercitatio anatomica de motu cordis et sanguinis in animalibus,*" printed in 1628.

Though René Descartes (1596-1650) in his book "*Traité de l' homme*" (completed 1637, published Paris 1664) was not able to agree completely with Harvey, for Descartes held that the heart was expanded by its innate heat, an expert mathematician, Giovanni Alfonso Borelli (1608-1679), was amongst the first adherents of Harvey's views on the circulation and attempted to "ornament and enrich by mathematical demonstrations" what Harvey had tried to calculate in a somewhat imperfect manner. Borelli is chiefly remembered as having suggested that the heart was moved by the action of nerves like other muscles, a view which modern investigations have somewhat modified; particularly since by means of artificial tissue cultures (explantation) of chick embryos it has been seen that heart muscle fibres will contract rhythmically before any nerve connection is formed.

Blood Coagulation.

During many centuries the opinion was held that clotting was the result of cooling the blood outside the vessels; a view no doubt induced by the observation of the freezing of water which is superficially similar. It was William Hewson (1739-1774) who proved that cold delays coagulation, which, moreover, can be inhibited by various other methods; he also described what we now know as "fibrinogen." Since then the problem has been assiduously investigated but the intricate mechanism of coagulation is not yet completely ascertained. The contemporary interpretation seems to be that the circulating blood contains fibrinogen, prothrombin and calcium ions; the prothrombin is protected from the action of the Ca by anti-thrombin or heparin present. When blood is extravasated, a cephalin-like compound with thromboplastic properties (a protein phospho-lipin complex) is set free, either as the result of injury to the tissues cells or following the breaking up of thrombocytes (blood platelets). This thromboplastic substance neutralises the heparin (antithrombin) and the Ca acts on the prothrombin, thrombin is produced, converts fibrinogen into fibrin and a clot is formed.

Respiration or Oxygenation of the Blood.

Robert Boyle (1627-1691) in his "*Memoirs for the natural history of the humane blood and the spirit of that liquor*" published in 1683 says in his preface ". . . . what is generally known of humane blood is as yet imperfect enough and consists much more of observations than experiments; being suggested far more by the phenomena that Nature herself has afforded physicians, than by tryals industriously made to find out what she will not, unsolicited by art, discover."

His "tryals" or experimental observations relate to the composition of the serum and the "spirit" (gases) of blood and may be considered in the light of pioneer investigations of the Chemistry of the blood; for he first experimented with flames and

animals *in vacuo* and demonstrated that air is necessary to combustion and life. Then in 1667 Robert Hooke (1635-1703), by blowing air briskly over the open thorax of a dog, showed that artificial respiration can keep the animal alive without any movement of chest or lungs. Further, about 1669 Richard Lower (1631-1690) injected dark venous blood into the insufflated lungs and concluded that its consequent bright colour was due to its absorbing some of the air passing through the lungs. Finally, John Mayow (1643-1679) demonstrated that the dark venous blood is changed to bright red by taking up a certain portion of air which he termed "igneo-aerial particles."

All these observations required for their understanding a more accurate knowledge of the components of air, which began with the discovery of oxygen in 1772 by Joseph Priestley (1733-1804) and was advanced by the recognition in 1775 of its true nature, by Antoine-Laurent Lavoisier (1743-1794); this knowledge was furthered by the constructive criticism of the mathematician Joseph Louis Lagrange (1736-1813).

It is worth recording that it was not until 1837 that the physicist Heinrich Gustave Magnus (1802-1870), employing a mercurial air pump, proved that both venous and arterial blood contained oxygen and CO_2; so that the development of the physiology of respiration, from Boyle to Magnus, can be said to be the summary of views of several different investigators, from the biological, chemical and mathematical aspects.

Blood Transfusion.

The vogue of venesection in the seventeenth and eighteenth centuries gave rise to many disquisitions on blood and also to the practice of transfusion, which though suggested by the physician and alchemist Andreas Libau [Libavius] (1546-1616), was first performed by Richard Lower (1631-1690) on animals. This procedure, as we all know, is referred to in Pepys' diary under the date November 14th, 1666: ("This did give occasion to many pretty wishes, as of the blood of a Quaker to be let into an Archbishop and such like.") Nevertheless it is common knowledge that the transfusion of blood for therapeutic purposes has only lately been rendered possible by knowledge acquired in relation to coagulation, the specificity of sera and different blood groups; for Landsteiner in 1901 classified three groups, whilst Lansky (1907) recognised four. Moss (1910) confirmed the latter grouping, which is now generally admitted. The summation of these modern researches have rendered transfusion first safe and then vitally important.

Blood Morphology.

If we then consider the minute elementary composition of the blood, we find that Jan Swammerdam (1637-1680) in his "*Biblia naturæ*" (published by Boerhaave in 1737) first described and

illustrated erythrocytes from the frog in 1658, whilst Malpighi in 1665 saw them in the mesentery of the hedgehog, but considered them to be fat droplets, whilst he gave an accurate description of capillary circulation. It was Antonj van Leeuwenhoek (1632-1723) who in a letter to the Royal Society in 1674 gave a full description of the erythrocytes of man, which then, by comparing with a coarse grain of sand, the eye of a louse and the thickness of a hair, he determined to have a diameter of approximately 8·7μ, which can be considered surprisingly accurate because contemporary measurements give 8·8μ as an average for the human red blood corpuscle in plasma. He then showed that though circular in mammals, erythrocytes were oval in birds, frogs and fishes and that the colour of blood was due in the first instance to the corpuscles and was not the colour of the fluid.

The microscopy of the blood remained in abeyance for considerable time after van Leeuwenhoek, but J. B. Senac (1693-1770) in 1749 refers to " globules blancs du pus " and it seems that it was again Hewson who in 1773 first clearly distinguished erythrocytes from leucocytes in the blood, though he appears to have considered that mammalian red blood cells are nucleated.

The third class of morphological elements of the blood, the thrombocytes or blood platelets appear to have been first seen by Alexander Donné (1801-1878) in 1842, whilst G. Zimmermann (1817-1866) in 1848 gave a more detailed description, but their significance remained a long time obscured by the presumption that they were forerunners of erythrocytes. They were further referred to by William Osler in 1873 and more fully and adequately in 1883 by Giulio Bizzozzero (1846-1901), who recognised that they played a part in the coagulation process.

The present view on the histogenesis of thrombocytes is due to J. Homer Wright, who considers them to originate from the breaking up of megakaryocytes.

Hæmatin crystals were first figured in 1830 by Sir Everard Home (1756-1832) and hæmoglobin first obtained and its derivatives spectroscopically detected in crystalline form in 1862-3 by E. F. I. Hoppe-Seyler (1825-1895) though even now the chemistry of hæmoglobin is far from being a closed chapter. Even a cursory review of the complex problems connected with the chemistry of the blood and their solution would oblige the net to be cast too wide for the present purposes.

Blood Generation and Regeneration.

The production of blood corpuscles or hæmatopoiesis is a word coined by J. Stam, who in 1743 wrote a book on " Sistens αἱματοποίησις." René Descartes (1596-1650) held the ancient view that the formation of the blood took place in the liver out of the chyle of food, but Fabricius and also Harvey were puzzled about the origin of the blood, which they had noticed in the early stages

of incubating eggs. Harvey asks: "What artificer can transform the two liquors into blood, when there is yet no liver in being?" This question was answered by Caspar Frederich Wolff (1733-1794) who discovered in 1759 the blood islands of the blastoderm of chick embryos, but a return to ancient opinions appeared to have taken place when the embryonal development of blood in the liver was observed by Weber and Kölliker in 1846.

Later on it was noted that the reproduction of blood took place in the bone marrow, as first described in 1868 by Ernest Neumann (1834-1918), let it be noted, in connection with morbid or leucocythæmic processes. Paul Ehrlich (1854-1915) established the origin of leucocytes from the normal bone marrow and by tinctorial methods distinguished the various categories of white blood cells.

Since the pioneer observations which Ehrlich began in 1877, progress has been continuous in relation to blood morphology and numerous comparative observations have been made connected with the blood generation during embryonal life and blood regeneration in adult life; these studies have resulted in the definite conception of blood as the composite result of the elaboration of various tissues, with several different vital functions.

No account of the development of the knowledge of hæmatology would be complete without some reference to the discussion relative to the monophyletic, dualistic and polyphyletic theory of blood formation or hæmocytopoiesis. It may be explained that Rudolf Virchow (1821-1902) held that connective tissue could be converted into blood cells, but these and similar views could not be maintained when Ehrlich (1885-1898) had distinguished different blood cells by means of accurate tinctorial methods and exactly recognised the development and approximate origin of leucocytes and erythrocytes. Further, Naegeli (1900) elaborated the concept of the myeloblast or cell peculiar to the marrow and capable of development into blood cells. As a continuation of these views one school held that the erythrocytes and leucocytes took their origin from a pluripotential cell in the bone marrow, the other that erythrocytes and leucocytes were produced by different tissues. Eventually discussions were connected more exactly with the determination of the actual point where the irreversible determination took place from a marrow cell into erythrocyte, granulocyte, lymphocyte, or monocyte.

The numerous writings of Arthur Pappenheim (1870-1916); Maximow (1902-10) and his pupils; of Adolfo Ferrata (1918) should be mentioned because they attempted to prove with extreme assiduity, by all means at their disposal, that lymphocytes and erythrocytes developed from the same marrow cell.

The latest contribution to the subject is that of Florence Sabin (1920-1928) and her collaborators and in my opinion it is if not conclusive, at least decisive in relation to several points. By means of experiments on pigeons, in which the fat in the marrow had

been reduced by means of fasting and comparisons with rabbits whose marrow was stimulated in various ways, it was demonstrated that the three principal cells of the pigeon's blood, *i.e.*, erythrocytes, granulocytes and megakaryocytes (or aggregated thrombocytes) are formed in the bone marrow; the lymphocytes in the lymph glands and the monocytes in the diffuse connective tissue. Such views do not agree with the observations obtainable by means of tissue cultures; it is not unfrequently the case that the results of experiments "*in vitro*" do not confirm those obtainable "*in vivo*."

What is now known as the "reticulo-endothelial system" was probably first recognised in 1884 by Elias Metchnikoff (1845-1916) and later more accurately investigated in 1900 by Louis Antoine Ranvier (1855-1922). Its extent and functions were defined by Aschoff and Landau (1913).

The attention claimed by the cellular elements of the blood, partly owing to their diagnostic significance, should not induce a neglect of the most important constituent of the blood, that is the plasma, which when deprived of fibrin is serum and the subject of immunology. Plasma consists mainly of water and the water metabolism of the blood is probably better known than that of tissues; the three principal recognised entities of the plasma: fibrinogen, globulin and albumin have also been accurately studied, but nevertheless it is not ascertained whether they are separately elaborated or whether any one can be converted into the others in the circulation. The first researches on the subject date from Claude Bernard (1813-1878), who in 1848 concluded that the plasma originated in the bowels and liver; though since then numerous investigators have approached the problem, yet the latest comprehensive paper on the subject, by Reimann, Medes and Fisher (1934) suggests that if the bone marrow and leucocytes are important sources of proteids in plasma they are not the only ones; that the precursors of blood proteins are incorporated in widely distributed cells, which the liver can then elaborate by disintegration, though various cells seem able to secrete blood proteins through their intact membranes.

Hæmatology is now being studied from so many aspects, not only as a medical discipline or speciality, but as a fully justified branch of biology, that it is impossible to review even in the most cursory manner the various advances made or to be expected in the many by-paths of the science.

In the compilation of these introductory remarks, acknowledgment must be made to the following works:—

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Moreover, the " Catalogue of the Surgeon General's Library," the " Dictionary of National Biography," and " Lexicon der hervorragende Aerzte" as also sundry articles in " The Encyclopædia Britannica " and other similar books of reference. Where possible, important authors have been verified in the original.

It is a great pleasure to be able to acknowledge the courteous and unfailing help of the Library Staff of the Royal Society of Medicine, who have obtained photostats of pages of rare works and confirmed references which were otherwise not accessible.

THE INCIDENCE OF TUBERCULOSIS AMONG CATTLE BELONGING TO THE WACHAGGA TRIBE IN TANGANYIKA TERRITORY (E. AFRICA)

By R. L. Cornell

Introduction.

The incidence of tuberculosis among native-owned cattle, which, throughout most of Tanganyika Territory, are of the Zebu race, appears to be extremely low. This statement is based on meat inspection returns and observations which are carried out at all the larger stations and townships, and reports of tuberculous lesions from such inspections do not amount to two per annum. The carcases so inspected are in general representative of the whole territory with the exception of animals belonging to the Wachagga people, and the animal management to which such cattle are subject is on the whole similar; that is, the animals graze in open range during the day under the care of herdsmen, and are confined at night in open thorn enclosed kraals to protect them from the attacks of carnivorous animals. In some districts housing at night may be in the family hut but free range during the day is general; calves, however, may spend some part of their early life in confinement.

Among the Wachagga, however, conditions are entirely different; this tribe inhabits the lower slopes of the Mountain of Kilimanjaro, a fertile but relatively densely populated and cultivated area. Their method of cattle management is peculiar in that the animals are confined throughout their lives in the owners' huts, are tied by the neck to stalls not dissimilar to those of a milking shed, and are hand-fed on cut fodder. The huts are high beehive shaped erections thatched with grass or banana leaves from the summit to the ground, and the only ventilation is through the door, which is closed at night. The owner with his family sleep on one side of the hut, and the cattle are stalled on the other; except for a few upright posts there is no division into two compartments. A ceiling of woven wattle is usually fixed about six feet from the floor. Drainage is primitive, the urine from the animals being led in a shallow gutter to the exterior of the hut; fæces are removed manually daily and are placed on the banana plantation which surrounds each family homestead. A small fire is often lighted in the hut, and the walls are impregnated with carbon and the atmosphere smoky. Fodder consists of wild grasses cut and transported from the plains below by the women. Every second day a banana tree is cut down and the stem and fronds sliced into small fingers and fed to the animals. The water content of this stem is extremely high and no additional water is allowed, the natives stating that if water in addition to banana is given diarrhœa results.

Chagga Hut and Native Cow.

The reasons underlying this somewhat peculiar animal management appear to be lack of sufficient grazing close to the homestead or village, tsetse fly infestation of neighbouring grazings and, in the past, fear of marauding and raiding neighbouring tribes.

On the eastern side of the mountain, which is less densely populated and where a greater range of "fly" free grazing exists, one finds gradation from complete confinement to complete open range management with open kraaling at night, the intermediate custom being for the animals to be chiefly stall-fed, but to go out for part of the day or for a few days a week to free range-grazing. These are usually animals from villages some little distance from the grazing lands. The number of animals kept in a single hut varies from two to six and are in the majority milch cows. Calves, however, of all ages are met with, and also an occasional bull which does service to his owner's cows and also to those of the neighbours. Oxen are rarely encountered so confined.

Under the somewhat peculiar environment described, the cattle fare better than might be expected. The animals are slow in growth and maturity, and even when mature are small and stunted; good bodily condition is, however, the rule rather than the exception, and some animals are fat. Milk production appears very low, even for native cattle. The skin is thin and soft, and the hair fine and short; the claws of the digits are often overgrown. Photophobia is shown when animals are brought outside the hut, as might be expected, but no evidence of blindness or ophthalmia was met with, contrary to what has been stated.

The photographs reproduced give some idea of the type of hut in which the cattle live, and the cow shown is a fully-grown animal and is characteristic of the type encountered.

The Wachagga are a meat-eating people but do not except under exceptional circumstances slaughter and eat their own stock. Their meat requirements are met by purchase of stock from neighbouring tribes at cattle sales which are held in the district, and it is for this reason that information regarding the incidence of tuberculosis among their own cattle is scanty.

The incidence of human tuberculosis in the tribe is high; phthisis, bone, joint and gland infections are met with and are the subject of special investigation and ameliorative effort on the part of the medical department. While there has been no positive evidence that the cattle have played any important part in the prevalence of the human disease, it appeared that a survey by means of the tuberculin test was desirable.

Tuberculin Tests.

Testing was carried out by the double intradermal method; a few check retests by the subcutaneous test were unsatisfactory owing to erratic pre-injection temperatures. Under field conditions in tropical countries the subcutaneous test is unsuitable or

impossible in operation, normal animals during the heat of the day often showing an irregular temperature curve, and it may be said that temperatures taken after about 9 or 10 a.m. can be entirely misleading. Further, under the conditions under which these tests were carried out, with small groups of animals widely spread over a considerable area, the recording of temperatures every three hours would have been impossible unless one had confined attention to only a few animals per day.

The animals tested may be divided into two groups—those belonging to natives of which one or more members of the family had shown acid-fast bacilli in the sputum (the records being kindly supplied by the medical department), and those cattle belonging to natives of which no medical history was known. It should be pointed out, however, that probably this second group of people was by no means free from tuberculosis. Many natives still do not avail themselves of the medical facilities offered, and from the known incidence of the disease in the district it is very probable that sputum-positive individuals could have been found here also. Calves of about three months old and over were tested whenever met with, as it was considered that infection in this group might furnish some indication of the presence of udder disease or milk infection. The results of the tests are set out in the following table :—

TABLE I.

	Animals belonging to natives of which some member of the family had shown acid-fast bacilli in the sputum.			Animals belonging to natives of unknown medical history. (76 animals kept under free range conditions are included in this group. All these gave negative results.)		
	Positive.	*Negative.*	*Doubtful.*	*Positive.*	*Negative.*	*Doubtful.*
Cows and heifers	2	97	8	5	170	9
Calves	–	41	–	1	65	1
Bulls and oxen...	–	9	–	–	32	1
Total ...	2	147	8	6	267	11

Total number tested : 441. Positive 8 (1·8 per cent.). Negative 414 (93·8 per cent.). Doubtful 19 (4·3 per cent.).

It may be here stated that all the results recorded as negative were clear cut, though the increase of skin measurements was greater than that usually encountered when testing European cattle in England, and the positives were equally definite. The number of doubtfuls may appear rather high. It was found, however, that very thin-skinned animals sometimes showed very large swellings, hard on palpation but with ill-defined edges and with a tendency to diffuseness around the margins, and these could only be classed as intermediate between a definite positive and a definite negative. All such doubtful reactions were retested

TABLE II.

	Date of Test.	*Skin.*	*First measurement.*	*Second measurement.*	*Remarks.*	*Result.*
1. White heifer calf about 6 months old	1st 18.12.33	3	6	10	Diffuse	Positive
	2nd 27.2.34	3	7	14	„	„
	3rd 17.3.34 O.T.	3	6	13	„	„
	glyc. broth	3	5	8	—	—
	S.B.T.	3	5	6	—	Negative
2. Aged cow	1st 7.2.34	4	9	22	Diffuse	Positive
	2nd 13.3.34	3	13	22	„	„
	3rd 24.3.34 O.T.	5	11	14		Doubtful
	glyc. broth	5	10	10	—	—
	S.B.T.	5	8	9		Negative
3. Brindle cow	1st 24.1.34	3	9	17	Diffuse	Positive
	2nd 13.3.34	4	9	13	„	„
	3rd 26.3.34 O.T.	4	10	13	„	„
	glyc. broth	4	·9	11	—	—
	S.B.T.	4	6	8		Negative
4. Black bull	1st 7.2.34	4	12	14	hard swelling	Doubtful
	2nd 13.3.34	4	9	14	Slightly diffuse at margins	„
	3rd 26.3.34 O.T.	5	9	12·5	„	„
	glyc. broth	5	6	11	—	—
	S.B.T.	5	5	8	—	Negative
5. Black and white cow (very thin)	1st 18.12.33	3	8	15	Diffuse definite œdema	Positive
	2nd 27.2.34	4	8·5	17	„	„
	Third test not carried out.					

O.T. = Old tuberculin, S.B.T. = Synthetic broth tuberculin.

after a minimum period of a month, but the same type of reaction recurred.

Four positive reacting animals and one doubtful reactor were obtained, retested and subsequently slaughtered and examined. The details of these tests are recorded; in the final test, injections with old tuberculin, synthetic broth tuberculin, and concentrated glycerin broth were made side by side on the neck.

Post-Mortem Examinations.

The bull recorded as doubtful to all the tests with old tuberculin was included for *post-mortem* examination as the reaction was typical of this class. In animals Nos. 1, 3, 4 and 5 no microscopic lesions of tuberculosis were detectable; smear examinations of lymphatic glands and tracheal mucus were also negative. Guinea-pigs were injected with suspensions of lymphatic glands, and when killed after periods varying from 47 days to three months appeared quite normal. Search was made for the so-called skin lesions which have been recorded in America, but none could be found. It is interesting to record that in the case of animal No. 1 (white heifer calf), the *post-mortem* was carried out 24 hours after the final injections of the tuberculins, and the area of skin under the double injection of old tuberculin showed congestion and thickening, while those under the synthetic broth tuberculin and concentrated glycerin broth showed no congestion and only slight thickening. Animal No. 2 (aged cow) showed, on *post-mortem*, near the anterior border of the apical lobe of the right lung, two adjacent lesions each about the size of a walnut. These lesions were enclosed in a thin but complete fibrous capsule and contained yellow caseo-calcareous material which easily shelled out from its capsule. No acid-fast bacilli could be detected in smears from these lesions, and a guinea-pig inoculated with a suspension of this material was healthy when killed 89 days later. No other lesions suggestive of tuberculosis could be found, and no macroscopic lesions were seen in the intrathoracic glands. The histology of the lung lesions in sections did not in any way resemble that of tuberculous tissue and suggested that they were degenerated cysts of a parasitic nature.

Discussion.

The retests on four of the animals recorded in Table II suggest that, had all the tests been carried out with synthetic broth tuberculin alone, none of the animals would have been recorded as positive. It is, however, evident that they showed sensitisation to old tuberculin; at the third test, however, all reactions were somewhat less pronounced than the first two, and this was attributed at the time to the relative short period between the last two tests, which was necessitated by force of circumstances. In a short series of tests of 34 head of cattle, using both old tuberculin and

synthetic broth tuberculin, it appeared that a few reactions rather more pronounced than a typical negative still occurred and caused some hesitation of mind as to their true interpretation.

The question of the true interpretation of reactions, bearing in mind the fact that larger swellings than those recorded in Great Britain appear to be the rule in this country, was considered from the study of the mean rise in skin measurement in the different categories. They are set out in the following table; figures obtained from herds tested previously, and in which there was little reason to suspect tuberculosis, are included for comparison.

Table of Average Mean Rise of Skin Measurement in Millimetres.

Cattle Tested.	*Negative.*			*Positive.*	*Doubtful.*	
	O.T.	*S.B.T.*	*G.B.*	*O.T.*	*O.T.*	*S.B.T.*
Chagga cattle kept in huts : infection with bovine bacilli unknown ; infection with human bacilli a possibility	6·3	3	5·7	12·2	10	6·3
Chagga cattle kept in open range, little reason to suspect sensitisation ...	5·5	—	—	—	—	—
Dairy herd, Friesian grade cattle, little reason to suspect infection or sensitisation	7	—	4	24 (1 case only, no lesions on P.M.)	11	—
Dairy herd, Ayrshire grade cattle, little reason to suspect infection or sensitisation	5·4	—	4·4	—	13 (1 case only on retest 8)	

O.T. = Old tuberculin. S.B.T. = Synthetic broth tuberculin. G.B. = Concentrated glycerine broth.

These records confirm the impression held when the tests were carried out that the mean rise in skin measurement was greater in all animals than that encountered in Great Britain, and while it is realised that no undue weight should be placed on measurement, they have helped to show that errors in interpretation cannot be accepted as an explanation of the no lesion reactors met with.

When the testing of cattle, many of which were being kept under circumstances which allowed them to take in tubercle bacilli of the human type, was first considered, the possibility was visualised that many reactors might occur but show no macroscopic lesions on autopsy, *i.e.*, the ingestion of human bacilli would render the animals allergic without producing marked lesions. In this event

the detection of infection with the bovine strain would be rendered difficult or impossible. Judging from the number of negative reactions encountered, this does not appear to have occurred to any great extent.

It is realised that failure to detect bacilli in smears or by inoculation of guinea-pigs is unsatisfactory evidence of their non-existence, but to attribute the reactions to old tuberculin of the four positive animals examined to sensitisation by human bacilli appears equally unconvincing, if one admits that a small percentage of anomalous results to the test do occur.

Shroeder (1923 and 1926), in discussing the problem of " no lesion " tuberculin reacting cattle, considers that infection with human bacilli is not infrequently an explanation of their occurrence and records an instance in which " the obtainable circumstantial evidence proved almost as conclusively as such evidence can, that fairly numerous "no lesion" reactions in a herd in which there should have been no reactions were due to an attendant affected with pulmonary tuberculosis." He further states that the sensitisation of cattle through their exposure to human bacilli is coupled with a number of peculiarities. The same exposure apparently does not sensitise all cattle; factors like individual susceptibility, the condition of the animal at the time of exposure and virulence of the bacilli seem to make considerable difference. Further, that cattle so sensitised react once with the subcutaneous test, but not again when retested.

Stenius (1933) records that in Finland, where bovine tuberculosis is rare and human infection not infrequent, the percentage of tuberculosis recorded in abattoirs is 0·1 per cent., while tuberculin tests gave figures of 2 to 3 per cent., and he explains this difference as being due to infection with human bacilli.

The question of the resistance of Zebu cattle to tuberculous infection may have some bearing on these results. The incidence of the disease in cattle of this race appears to be very low wherever they occur throughout the world, and lesions when encountered are generally confined to glands and are calcified and non-progressive in type.

In Tanganyika, as has been stated, its occurrence in meat animals is rare; similar conditions appear to exist in the Gold Coast.

In the report of the Anglo-Egyptian Sudan for the year 1932 it is stated that " Cases of bovine tuberculosis come to notice but rarely," and, as the Veterinary Research Officer points out, in consideration of the number of cattle slaughtered in controlled centres for food and of the fact that several hundred cattle yearly are examined *post-mortem* in the veterinary laboratories at Khartoum and Malakal, the disease must be very uncommon.

In Uganda there is a marked contrast between the incidence of tuberculosis in native cattle of the Zebu race and in native cattle

of the long horn humpless Ankole race, which appears to have sprung from essentially different parent stock, the percentage of tuberculosis among the latter being as high as 12·5 per cent.

In Indo-China, Vittoz and Souchand point out the extreme rarity of the disease.

In India also this appears to be the general rule, and the question of the virulence of the infecting strains and the resistance of the cattle has received the attention of Sheather, Edwards and Soparker. Inoculation experiments carried out by Soparker showed that strains isolated in India were of virulence equal to the usual European ones. They also showed that some individual native calves showed greater resistance to subcutaneous inoculation than is the rule in Europe. In this connection it appears reasonable to suppose that such test inoculations are too drastic to bring out such slight differences of immunity as might be sufficient to prevent or retard natural infection.

The extent and disposition of tuberculosis in Zebu cattle as indicated from reports and abattoir examinations has been reviewd by Hornby in 1933 in a paper presented to the British Medical Association of East Africa, and he concludes by saying: "a reasonable view seems to be that native races of cattle, particularly the Zebu, are more resistant to tubercle infection than are the cattle of European race and that the more open air life led by the majority of native cattle further contributes to a low incidence of tuberculosis."

This, in the absence of direct proof to the contrary, appears to be a probable hypothesis to explain the low incidence found and may be an explanation of the fact that Chagga cattle apparently do not allow human bacilli to enter their tissues and sensitise them to tuberculin.

Conclusions.

(1) Bovine tuberculosis among native cattle (Zebu) of the Wachagga is extremely low.

(2) The double intradermal test was applied to 441 animals with positive reactions in eight instances (1·8 per cent.). No lesions of tuberculosis were discoverable in four positive reactors and one doubtful reactor slaughtered; moreover, guinea-pigs inoculated with material from the lymphatic glands were also negative.

(3) Bovine tuberculosis is a negligible factor in the incidence of tuberculosis among the human population.

(4) No positive evidence could be obtained that the ingestion or inhalation of tubercle bacilli of the human type sensitised the animals to tuberculin or was the cause of the reaction in those animals which reacted positively to the test, although it appears probable that such ingestion had taken place.

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OBITUARY

PROFESSOR S. H. GAIGER, F.R.C.V.S.

It is with profound regret that we have to record the death of Professor Gaiger, at Birkenhead, on December 14th. It was known that about a year ago he underwent a serious operation, but it was understood that he had made an apparently complete recovery. The announcement of his death has therefore come as a great shock, and as a defeat of the very earnest hopes entertained by his numerous friends.

Professor Gaiger had steadily risen to a very high place in the estimation of those who were brought into contact with him, and who were able to judge of the sterling character of his work in the subject to which his life was devoted—the advancement of knowledge in the sphere of veterinary pathology; his death creates a very notable blank in the list of those who pursue that subject.

His research work was marked by a wide general knowledge, by insight, and by caution in interpreting results. The most important of his researches were those connected with the ætiology and prevention of braxy and lamb dysentery. He also found time to write a text-book on Veterinary Pathology and Bacteriology, which formed a valuable addition to the works on that subject.

Even in this short biographical note it would be an omission not to mention the fact that Professor Gaiger himself suffered from glanders as the result of infection when making a *post-mortem* examination of a pony in India, and recovered from the disease after nearly two years. The fact is mentioned because of the great courage which he displayed under this dire misfortune. Throughout the period of his illness he often suffered pain, amounting to torture, not only from the effects of the disease, but also from the after-effects of the surgical operations which had to be performed. Not counting minor incisions, the operations numbered 45, of which 27 were done under a general anæsthetic, and one of them was the removal of an arm. Other men have contracted glanders and recovered, but surely none experienced so much suffering or bore it with such heroic fortitude.

His death is all the more to be lamented because he has been cut off at the height of his powers, and all who knew him had high expectations of further achievements by him in research.